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Synthesis of fragments of the capsular polysaccharide of Neisseria meningitidis (serogroup A) suitable for bioconjugation

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Synthesis of fragments of the capsular polysaccharide of *Neisseria meningitidis* (serogroup A) suitable for bioconjugation

A Thesis presented for the Degree of Doctor of Philosophy

By

Alan Black

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CONTENTS

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DECLARATION

I hereby declare that the research described herein has been carried out and that this thesis is of my own work. This thesis has not been accepted in fulfillment of the requirements of any other degree or qualification. The research described herein was carried out in the College of Life Sciences, at the University of Dundee, under the supervision of Dr. A. V. Nikolaev.

Alan Black

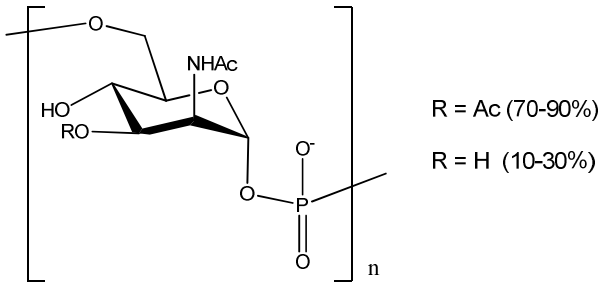
CERTIFICATION

I hereby certify that Alan Black, BSc Hons, has undertaken full-time research at the College of Life Sciences, University of Dundee, under my direction since October 2007, and that he has fulfilled the conditions of ordinance 14, so that he is qualified to submit this thesis for the degree of Doctor of Philosophy.

Dr. Andrei V. Nikolaev

SYNOPSIS

One of the causative agents of meningitis is the gram-negative bacteria *Neisseria meningitidis*. This is a fairly common bacterium, which is present in the noses and throats of 10-15% of the population but rarely causes serious disease. The potentially fatal invasive disease occurs when the bacteria penetrates the mucous membrane of the nose and spreads to other parts of the body through the blood stream. In the region known as the meningitis belt, *N. meningitidis*serotype A is endemic in the population and is also known to cause large scale epidemics such as in 1996 when 250 thousand cases were reported leading to approximately 25 thousand deaths. An important virulence factor of this bacterium is the presence of a capsular polysaccharide, which also protects against phagocytosis. The capsular polysaccharide of serotype A is (1-6)-linked poly(2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate):

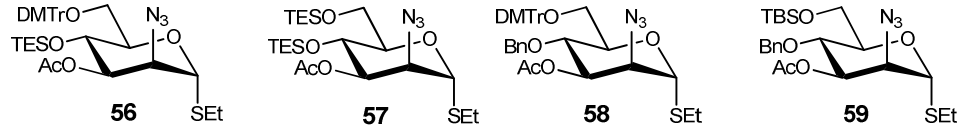


One of the issues with naturally derived carbohydrate polymers used in vaccine preparations is that they are heterogeneous mixtures that may include contaminants and unwanted impurities. Alternatively, through the use of synthetic carbohydrate structures, which can be chemically produced as single compounds, batch to batch variability can be eliminated and provide the desired CPS fragments in a more reproducible and robust manner. The chemical synthesis of CPS fragments also provides the opportunity to

incorporate a linker moiety into the structure allowing for conjugation to an immunogenic carrier protein.

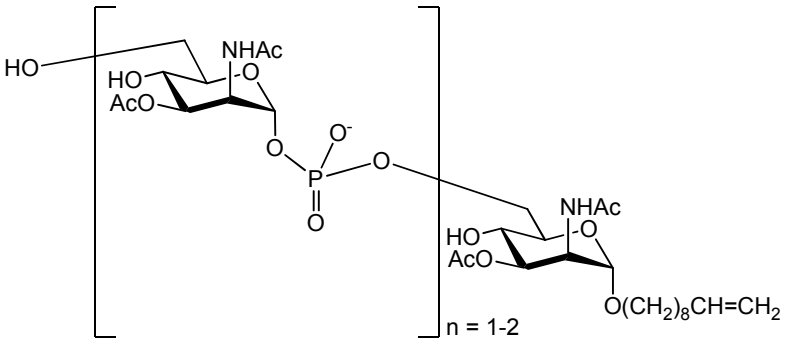
Therefore an efficient and reproducible method for the chemical synthesis of fragments of the capsular polysaccharide of *N. meningitidis*(serotype A) suitable for use in a potential glycoconjugate vaccine against this disease is highly desirable. To this end, we designed and chemically prepared *N. meningitidis*serogroup A synthetic phosphoglycans containing linkers, which allow further bioconjugation with a protein carrier.

Formation and elongation of the synthetic phosphoglycan chain proceeds through the condensation of a suitable anomeric H-phosphonate derivatives. In order to accommodate this all the monosaccharide synthetic precursors (compounds **56-59**) allow for hydrolysis of the O-1 position to the hemiacetal. This is then converted to the H-phosphonate before coupling to a monohydroxyl unit to form a phosphodiester linkage. Removal of the temporary protection at the O-6 position then gives the free hydroxyl needed for elongation to continue. To explore the optimal combination for the permanent O-4 protection and the temporary O-6 protection four different orthogonally protected monosaccharides **56-59** were designed.



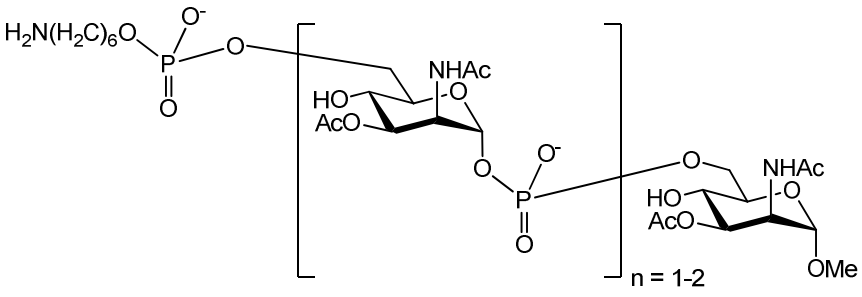
During the initial synthetic strategy a dec-9-en-1-yl linker was incorporated into the primary saccharide unit at the reducing end of the molecule. Chemical synthesis of the

'decenylated' phosphoglycans was attempted. They were synthesised as 'protected' derivatives.



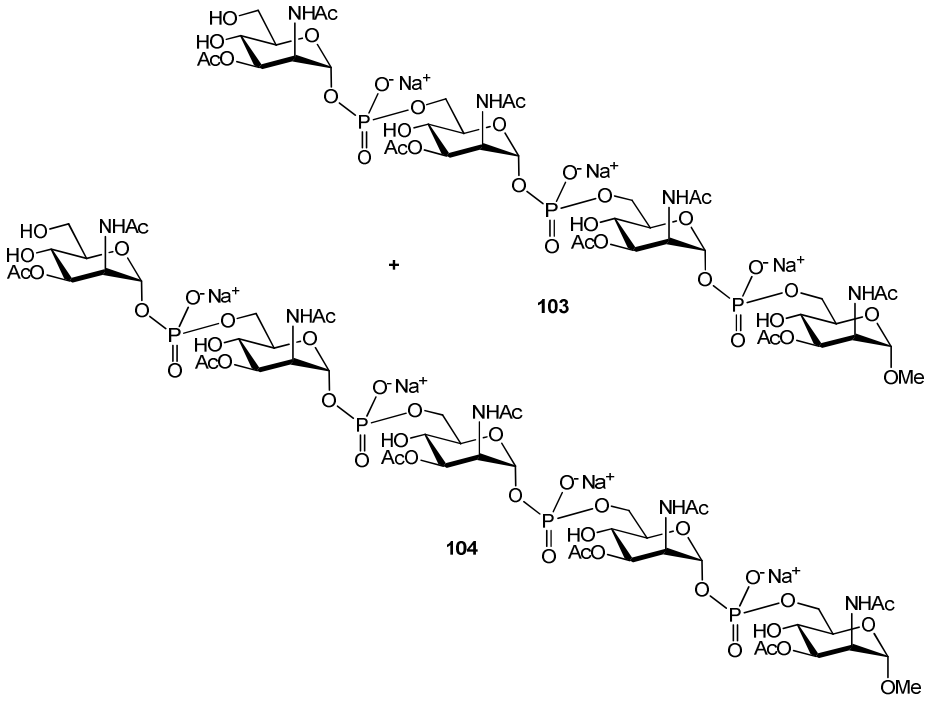
Complications arising during final deprotection of the decenyl containing fragments however made it clear that this was not suitable for this approach.

A slightly different approach was then used which would allow the building of the phosphoglycan fragments to the required size before finally capping the structure with a suitable 6-aminohexyl linker via a phosphate group at the non-reducing end of the phosphoglycan chain. This incorporated another phosphate moiety into the CPS fragments while also providing an amino group for bioconjugation.



In addition, we synthesised a few *N. meningitidis*serogroup Aphosphoglycan molecules without a linker moiety. The chain elongation was successful in the integration of up to 3 intersaccharidic phosphates in the molecule (which is a record for *N. meningitidis*serogroup Aphosphoglycans so far). Also the introduction of a fourth intersaccharidic phosphate was in part successful, but the reaction did not go to completion and resulted in an inseparable mixture of the corresponding pentasaccharide

tetraphosphate and the tetrasaccharide triphosphate as the starting material. After the global deprotection a mixture of the phosphoglycans **103** (with 3 intersaccharidic phosphates) and **104** (with 4 intersaccharidic phosphates) was isolated.



CHAPTER 1

1. INTRODUCTION

1.1 BIOLOGICAL INTRODUCTION

1.1.1 Meningitis

Meningitis is an acute life threatening disease brought on by swelling in the protective membranes lining the brain and spinal cord known as the meninges. The meninges are comprised of three membranes that, along with cerebrospinal fluid, enclose and provide protection for the brain and spinal cord, collectively called the central nervous system (see **Figure 1**).

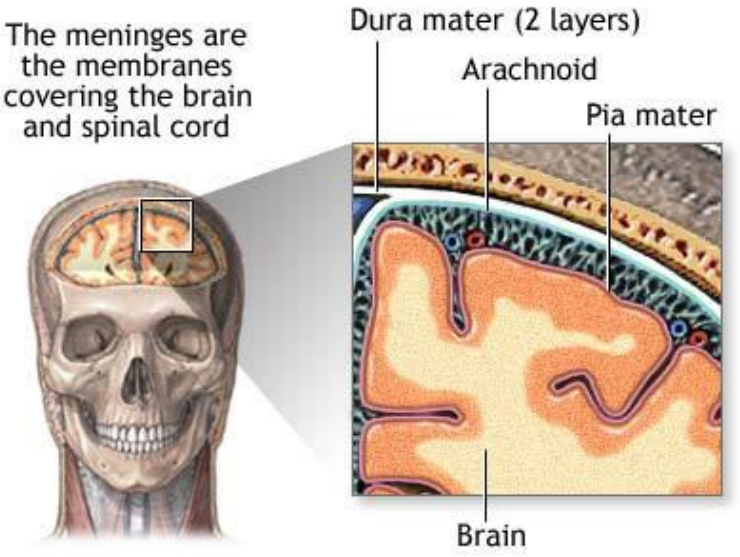


Figure 1: Meninges, protective membrane linings of the brain and spinal cord.[1]

The first membrane is the pia mater, a thin and very delicate impermeable membrane that adheres firmly to the brain. The second is the arachnoid mater, a more loosely fitting membrane, and as the name suggests has a spider web like appearance. The space between the pia mater and the arachnoid mater is known as the subarachnoid

space which is occupied by cerebrospinal fluid. The third and outermost membrane is the dura mater, the thickest and most durable of the membranes attached to both the inside of the skull and the arachnoid mater.

The swelling can be caused by several microorganisms, including bacteria, viruses, parasites or fungi and requires prompt medical attention, and owing to the proximity to the central nervous system is classified as a medical emergency.[2] Initial common symptoms of the disease in adults include severe headache, nausea, vomiting and the inability to flex the neck forward due to increased muscle tone and stiffness (known as nuchal rigidity).[3] For diagnosis the classic triad of symptoms is sought, which are nuchal rigidity, sudden fever and an altered mental status. This is problematic however as all three features are only seen in 44-46% of bacterial meningitis cases.[3,4]If none of the three signs are seen then meningitis is extremely unlikely.[4] Sensitivity to light (photophobia) and intolerance to loud noises (phonophobia) are other commonly associated symptoms. Unfortunately small children often do not display the aforementioned symptoms and may only appear irritable and unwell.[5] Left untreated, bacterial meningitis is almost always fatal while viral meningitis on the other hand tends to resolve spontaneously and fatality occurs only on rare occasions. With treatment the fatality rates associated with the disease vary widely but can be as low as 2% in infants and children and as high as 20-30% in neonates and adults.[6] Even with the successful treatment of children there may be lasting damage to the central nervous system, which is seen in approximately 15% of survivors. This may give rise to potential disabilities including sensorineural hearing loss, epilepsy, learning and behavioral difficulties along with a decrease of intelligence,[5] although the hearing loss may in some cases be reversible.[7]

1.1.2 *Neisseria meningitidis*

Neisseria meningitidis, commonly referred to as meningococcus, is a parasitic gram negative bacteria that was first described in 1884 by Marchiafava and Celli as an intracellular oval micrococci in a sample of cerebrospinal fluid (CSF).[8] A few years later in 1887 Anton Weichselbaum isolated the bacteria and managed to distinguish Pneumococci from meningococci in a sample of CSF and named it as *Diplococcusintracellularis meningitidis* (see **Figure 2**).[9] The first clinical descriptions of *Neisseria meningitidis* or meningococcus occurred a lot earlier following an outbreak of meningitis initially in 1805 in Geneva and soon after in New Bedford in 1806, although the causative agent was not known at the time.[10,11]

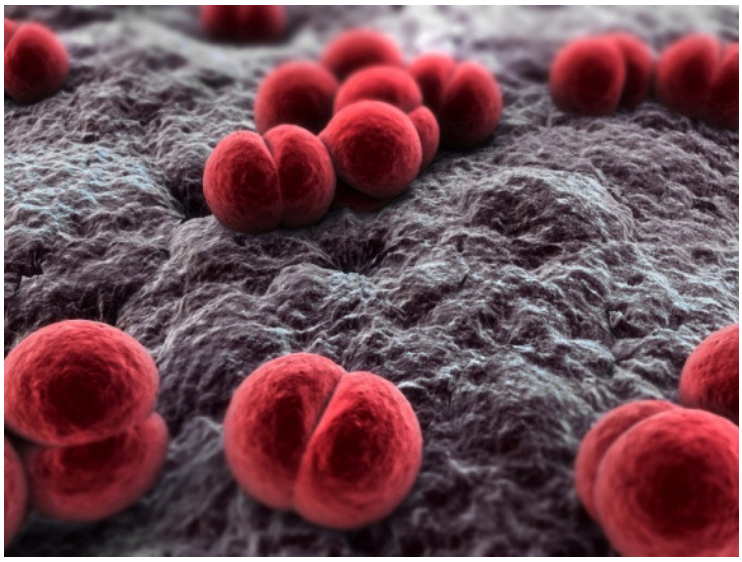


Figure 2: *Diplococcusintracellularis meningitidis*(*Neisseriameningitidis*).[12]

Interestingly, humans are the only known host of *N. meningitidis*. The most common natural habitat for the meningococcus is the epithelium of the naso- and posterior pharynx with 10-15% of the population being asymptomatic carriers of the bacteria which translates to hundreds of millions of carriers worldwide, with young

adolescents being the principal reservoir.[13] The frequency and pattern of disease, however, varies significantly between different regions of the world. In temperate regions the pattern seen is usually that of endemic disease with an annual incidence of between 1 and 10 per 100,000 population.[14,15] This is also subject to seasonal variation with most cases appearing in the winter months.[16] There can also be hyperendemic periods, with disease incidence up to 20 per 100,000, usually brought about following the appearance of new virulent clones into non-immune populations.[17,18,19] The story in developing nations however is quite different. Not only is there endemic disease but also regular outbreaks of epidemic meningococcal disease, where annual incidence rates erupt to 200 cases per 100,000 population. These epidemics occur in 5-10 year cycles and appear exclusively in the dry season.

Colonisation of the mucosal surfaces in the upper respiratory tract by *N. meningitidis* is initiated largely through contact with respiratory droplets or secretions from an infected individual. After acquisition of *N. meningitidis* to the upper respiratory tract the infection may stay asymptomatic or may infrequently result in local inflammation, invasion of the mucosal surfaces, possible access to the blood stream leading to sepsis (meningococcal septicaemia) and meningitis.[20] It should be noted though that internal infection is not part of the meningococcal transmission cycle and occurs more as an accident as crossing into the blood stream can be seen as a “one way ticket” for the bacteria. Once in the blood the meningococcus can no longer be transmitted through the usual route of respiratory droplets from coughing or sneezing and are in effect trapped in the host. In fact the relationship between virulence and transmissibility in the life cycles of many pathogenic organisms is still under debate.[21] The virulence of *N. meningitidis* is mediated by meningococcal structures and has been studied more intensively than transmissibility but the key event in both scenarios is the adhesion of the bacteria to the epithelial cells allowing the colonisation of the

nasopharynx. Pili and adhesins help facilitate adhesion and ultimately colonisation, while the bacterial capsule appears to prevent close contact with the membranes interfering with adhesion and will in effect reduce colonisation.[22] In fact, during studies of nasopharyngeal colonisation by meningococcus the down-regulation of the capsule is selected or it may even be switched off completely.[23,24] The capsule however must ultimately re-appear, which may assist the shedding of the bacteria from the epithelium, in order for the successful transmission to another host.[25]

1.1.3 Bacterial capsule

A principal component of most pathogenic bacteria, including *N. meningitidis*, is the presence of a capsule (see **Figure 3**). The structure of the capsule is in most cases a high molecular weight polysaccharide, although is some cases the capsule consists of a polypeptide.

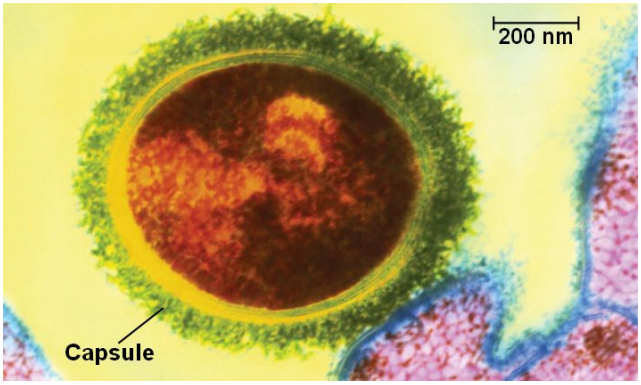


Figure 3: Diagram showing capsule surrounding cell surface.[26]

The capsule surrounds the entire surface of the bacteria attached to the outer cell wall and provides several functions. The production of a capsule is a major virulence factor that helps the bacteria evade clearance from the site of infection by providing protection from the host's immune response. The presence of the capsule also protects

the bacteria from phagocytosis, by hindering detection from opsonising antibodies, by the hosts defence cells. The capsule is in many cases hydrophilic giving the ability to retain water and prevent desiccation of the cell as well as providing anti-adhesive properties such as steric hindrance and repulsion through negative charge (e.g. sialic acid or phosphate).

The invasive strains of meningococcus express a polysaccharide capsule with a range of chemical structures. The exact chemical composition of the capsule was used originally to distinguish between different strains giving rise to what are known as different serogroups.[27] Currently there are thirteen structurally distinct serogroups that are known of which only six, designated A, B, C, Y, X and W-135, account for the majority of the invasive disease.[20,28]In fact, carriage strains are more widespread in nature than the disease causing strains hence *N. meningitidis* is considered an occasional pathogen. It is known that capsular polysaccharide expression is increased on clinical isolates derived from patients suffering invasive disease while the capsule is down-regulated on meningococci sampled from the nasopharynx. This was seen clearly during the Stonehouse meningococcal outbreak in Gloucester where the strain B:15 was isolated which showed the same genotype and phenotype with the invasive isolates encapsulated and the carrier isolates non-encapsulated.[29]

In the case of serogroups Y and W-135 the capsular polysaccharide structures are composed of alternating disaccharide repeat units of sialic acid and D-glucose or D-galactose respectively. Serogroups B and C exist as a homopolymer of N-acetylneuraminic acid with either an$\alpha 2\rightarrow 8$ or $\alpha 2\rightarrow 9$ linkagerespectively.The serogroup A capsule is composed of (1-6)-linked N-acetylmannosamine-1-phosphaterepeat units containing some 3-O-acetates, while serogroup X produces a phosphoglycan polymer of (1-4)-linked N-acetylmannosamine-1-phosphate (see **Figure 4**).

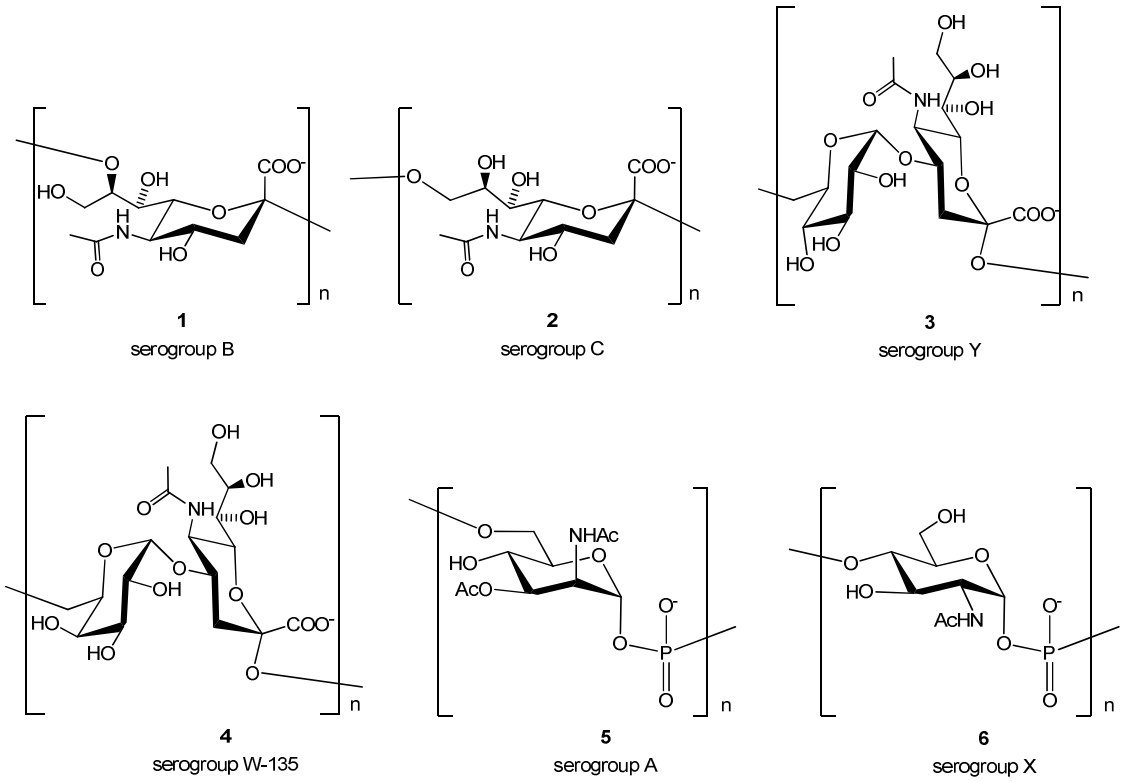


Figure 4: Repeat units of pathogenic *N. meningitidis* Capsules

1.1.4 *Neisseria meningitidis* serogroup A

The earliest studies of the capsule of *N. meningitidis* serogroup A showed that it contained both phosphorus and nitrogen but it was not until 1971 that it was shown that the polysaccharide was a polymer of partially O- and fully N-acetylated mannosamine phosphate.[30]Yet further study using ^{13}C nuclear magnetic resonance (NMR) was required to resolve that there was a (1→6)-phosphodiester linkage between the α-(N-acetyl-D-mannosamine) units, while also showing a 70% O-acetylation at the C-3 position.[31] Later detailed analysis, again using NMR, confirmed that the capsular polysaccharide of serogroup A was a homopolymer of (1-6)-linked poly(2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate)(see **Figure 5**) again with O-acetylation at 70-95%.[32,33] A small percentage of O-acetylation was also observed at the C-4 position, but it is possible this was due to acetyl migration from the C-3 position.

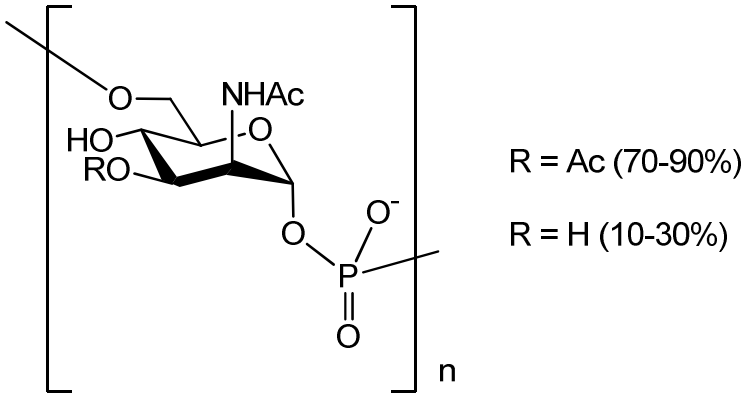


Figure 5: Repeating unit of *N. meningitidis*serogroup A capsule

The degree of O-acetylation at the C-3 position of the purified polysaccharide does appear to vary depending on the strain used and the isolation and purification techniques used, however, it is still regarded as an important immunogenicity factor for the polysaccharide.[34]

The ability of *N. meningitidis* serogroup A to cause large scale epidemics is still of major concern. Epidemic meningitis, usually caused by serogroup A, is greatly feared in sub-Saharan Africa as it causes death, disability and has been shown to be an important factor of poverty for the affected families.[35]In the region from Senegal to Ethiopia known as the meningitis belt(see **Figure 6**), *N. meningitidis* is endemic in the population and during epidemics up to 2% of the population can be affected.

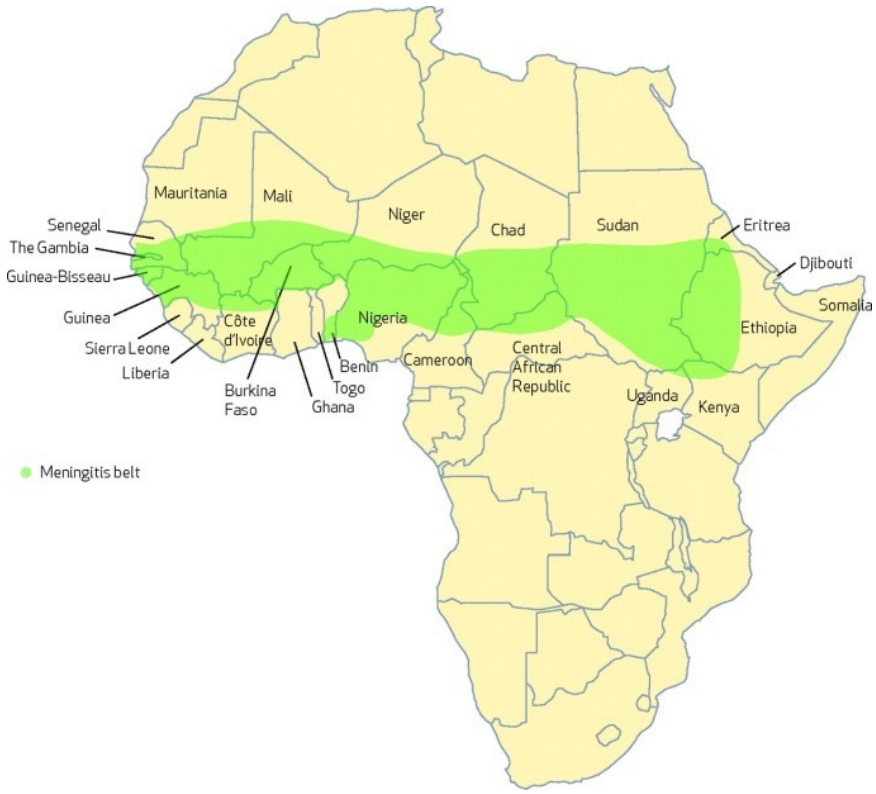


Figure 6: The African meningitis belt.[36]

The meningitis belt was first described by Lapeyssonnie in 1963, a vast area stretching almost 4 thousand miles, with a population of more than 300 million where major epidemics of meningococcal meningitis occur regularly.[37] He showed that there is a distinct periodicity for meningitis epidemics, which usually start at the beginning of the dry season in late December and promptly cease following the first rains of May or June. This epidemic season is characterized by a hot dry wind that generates a great deal of dust known as the Harmattan.[38] Other environmental factors that damage or weaken the integrity of the nasopharyngeal mucosa such as smoking, low humidity, cramped living conditions and even co-infections also increase the incidence of meningococcal disease.[20,39,40] Most countries within the meningitis belt are subject to large outbreaks in cycles every 5-12 years since the 1940's, however in recent years the intervals between epidemics has become irregular. One of the largestrecorded outbreaks was seen in 1996 when 200 thousand cases were reported which lead to 20 thousand deaths.[41]

Similar cycles of epidemic meningococcal disease have also been described in Asia, mostly in China, during the past century.[42] Studies carried out using strains available over the last 40 years have highlighted the origins of two meningococcal pandemics in China.[43] The first meningococcal serogroup A pandemic occurred in the mid 1960's and extended to Moscow and Norway in 1969, followed by Finland and Brazil in the early 1970's. The second appeared in China and Nepal in the early 1980's before spreading to Africa and the Middle East and most likely to India as well.[43,44]

1.1.5 Meningococcal vaccines

The first trial vaccines against *N. meningitidis* were developed over one hundred years ago in the early 1900's.[45] These early vaccines were composed of heat-killed whole cells, which were grown in culture, gently heated to around 60^{o}C to kill but not lyse the cells, before dilution to the required concentration for injection into the patient. These initial vaccines were not very effective showing variable efficacy and an array of severe adverse reactions. The greatest efficacy observed was 87% in a study carried out on volunteers from Camp Funston, Kansas, following an epidemic outbreak of meningitis at the camp. The vaccine was prepared locally using fresh clinical isolates of *N. meningitidis* serogroup A taken from asymptomatic carriers at the camp.[46] These types of studies were repeated but the level of efficacy seen in the Camp Funston trial was not reproducible.

It was later in the 1930's that a different approach was taken when Ferry and co-workers along with Kuhns and co-workers investigated a possible meningococcal exotoxin.[47,48,49,50] This was an attempt to repeat the success in the development of vaccines using tetanus toxoid and diphtheria toxoid, both utilising a detoxified exotoxin excreted by the bacteria. In these tests the supernatants derived from broth cultures of meningococci were investigated for their vaccine potential and they were able to show a

possible toxin-mediated immunological effect. Possibly, due to the presence of capsular polysaccharide among other agents in the supernatant cocktail. They were also able to show a reduction in meningococcal disease in young men who were vaccinated with this toxin but unfortunately were unable to prove either immunogenicity or efficacy and the exotoxin approach was abandoned.[51]

A few years later, during the Second World War, there were multiple outbreaks of meningococcal disease caused by serogroup A and C amongst army recruits. This prompted the further investigation of purified capsular polysaccharide as a possible vaccine, which had been successfully isolated in 1935.[52] Interest had grown around the potential of capsular polysaccharide as a vaccine candidate after it was demonstrated that anti-serogroup A meningococcal horse serum would prevent disease but only in proportion to the amount of anti-polysaccharide antibody it contained. The earliest of these immunological studies using purified serogroup A and C capsular polysaccharide however proved unsuccessful. This may have been due to the production techniques available at the time which could have allowed enzymatic degradation of the polysaccharides.[53]

The first major breakthrough in meningococcal vaccinations came in the 1960's when Gotschlich and co-workers were able to demonstrate the importance of the anti-polysaccharide antibodies for both serogroup A and C in the human immune response.[54,55]Their success came after they were able to develop a novel method in which they employed the cationic detergent Cetavlon to precipitate the capsular polysaccharides from whole culture. This resulted in highly purified, high molecular weight (greater than 100,000) capsular polysaccharide.[56] With this new method in hand they then went on to show that the capsular polysaccharide isolated in this way was immunogenic in adult volunteers.[57] The success continued in subsequent clinical trials that were carried out during serogroup C outbreaks on army bases. These showed that

the army recruits that were vaccinated with the capsular polysaccharide demonstrated reduced acquisition rates compared with unvaccinated recruits along with an estimated 90% efficacy for prevention of disease within the first 8 weeks of vaccination.[58,59]

There are currently two polysaccharide vaccines that are in routine clinical use. They are a bivalent serogroup A and C vaccine or a tetravalent serogroup A, C, Y and W-135 vaccine. These vaccines are routinely used to combat meningococcal serogroup A and C outbreaks and epidemics as well as a preventative measure in high risk groups such as military recruits, patients with immunodeficiencies and travellers to high risk areas. That being said there are still a couple of major disadvantages to the use of these vaccines. The first being that the purified polysaccharide vaccine itself is poorly immunogenic when given to infants and young children, the exact group who are most vulnerable to the disease.[60,61] Secondly, the duration of the immunity imparted by the vaccine is unfortunately quite limited and varies with age. In children 3 years of age or younger the vaccine provides almost no protection beyond 12 months following the vaccination.[62]

The poor immunogenic response gained from all polysaccharide antigens can be attributed to the fact that the immune response they induce is of a T-cell independent nature. Without the recruitment of the T-cells there is a lack of induction of immunological memory. Another problem is that repeated doses of these polysaccharide antigens induces an abnormally low response, it has been suggested that a possible reason for this being that the pool of polysaccharide specific memory B-cells is depleted.[63,64]

1.1.6 Glycoconjugate vaccines

It is possible to induce a T-cell dependent response with a polysaccharide antigen as long as the polysaccharide is first chemically conjugated to a protein carrier. This conjugate is then able to recruit the help of T-cells in the immunologic response. The success attributed to this technique can be seen clearly in the production of the *Haemophilus influenza* type b vaccine. The use of which has almost eradicated the disease in countries which have chosen to add it to their infant vaccination schedule.[65] This conjugation approach works best when the polysaccharide is conjugated to a strongly immunogenic protein. In the case of the *H. influenza* b vaccines either tetanus toxoid or diphtheria toxoid has been used as a protein carrier which has been proven safe and immunogenic.[66] These toxoids are exotoxins excreted by bacteria, which have been detoxified either by heat or chemical alteration but still elicit a potent immunological response. Meningococcal glycoconjugate vaccines are available and are produced from partially hydrolysed capsular polysaccharides that are size fractionated before chemically conjugating to a carrier protein. Vaccines produced this way have been subjected to immunogenicity studies and have proven successful at producing antibodies in infants and older children that are likely to be protective.[64,67,68,69]For example a *Neisseria meningitidis* group C (Men C) conjugate vaccine (Menjugate®) was introduced into the UK vaccination schedule for infants in 1999, along with a catch-up campaign with a single dose of vaccine given to 1 to 18 year olds. This resulted in a dramatic reduction in Men C meningitis and led to a major decrease in the circulation of Men C even in the unvaccinated population (herd immunity).[70] This decrease demonstrated that as well as providing protection from the invasive disease, vaccinated individuals had decreased asymptomatic carriage of Men C resulting in an overall reduction in the prevalence of Men C meningitis in the population.

Three quadrivalent meningococcal serogroup A, C, Y and W-135 conjugate vaccines are also available internationally which differ only in the protein carrier used. The first conjugated to diphtheria toxoid (Menactra™)[71], the second conjugated to CRM_{197}(Menveo™)[72] while the third is conjugated to tetanus toxoid (Nimenrix™)[73]. Unfortunately all remain too expensive for a mass vaccination campaign in Africa where the cost per dose needs to be around 50 cents.

I response to this the World Health Organisation (WHO) in partnership with the Program for Appropriate Technology in Health (PATH) set up the Meningitis Vaccine Project (MVP) in 2001. The aim of which was to eliminate epidemic group A meningitis by developing, testing and introducing a group A meningococcal conjugate vaccine which would be affordable in Africa.[74]This resulted in the release of MenAfriVac™ in 2010, a Men A – tetanus toxoid glycoconjugate vaccine,which can be produced for 44 cents per dose[75], and since rolling out more than 217 million people in 15 countries have received the vaccine.

One of the remaining issues for the Men-A glycoconjugate vaccine however is its relative stability for shelf life and storage. This is not an issue in developed countries where access to fridges and freezers are readily available as meningococcal vaccines in a lyophilised state can be stored for two years if kept cold. Access to cold storage in the developing world however is much more difficult to guarantee, especially in remote areas where vaccination takes place.Meningococcal polysaccharides, especially Men-A polysaccharides, are unstable at ambient temperatures due to depolymerisation which severely reduces the vaccines efficacy. To date the degree of polymerisation required in the meningococcal polysaccharide to induce an acceptable immune response remains unknown. To address this we designed a synthetic strategy to produce a set of Men-A capsular polysaccharides of varying length with linkers suitable for bioconjugation. After bioconjugation these could potentially be used in immunological studies to assess

the immune response induced from relatively small synthetic fragments, and from this address whether a fully synthetic route to a Men-A glycoconjugate vaccine is theoretically possible.

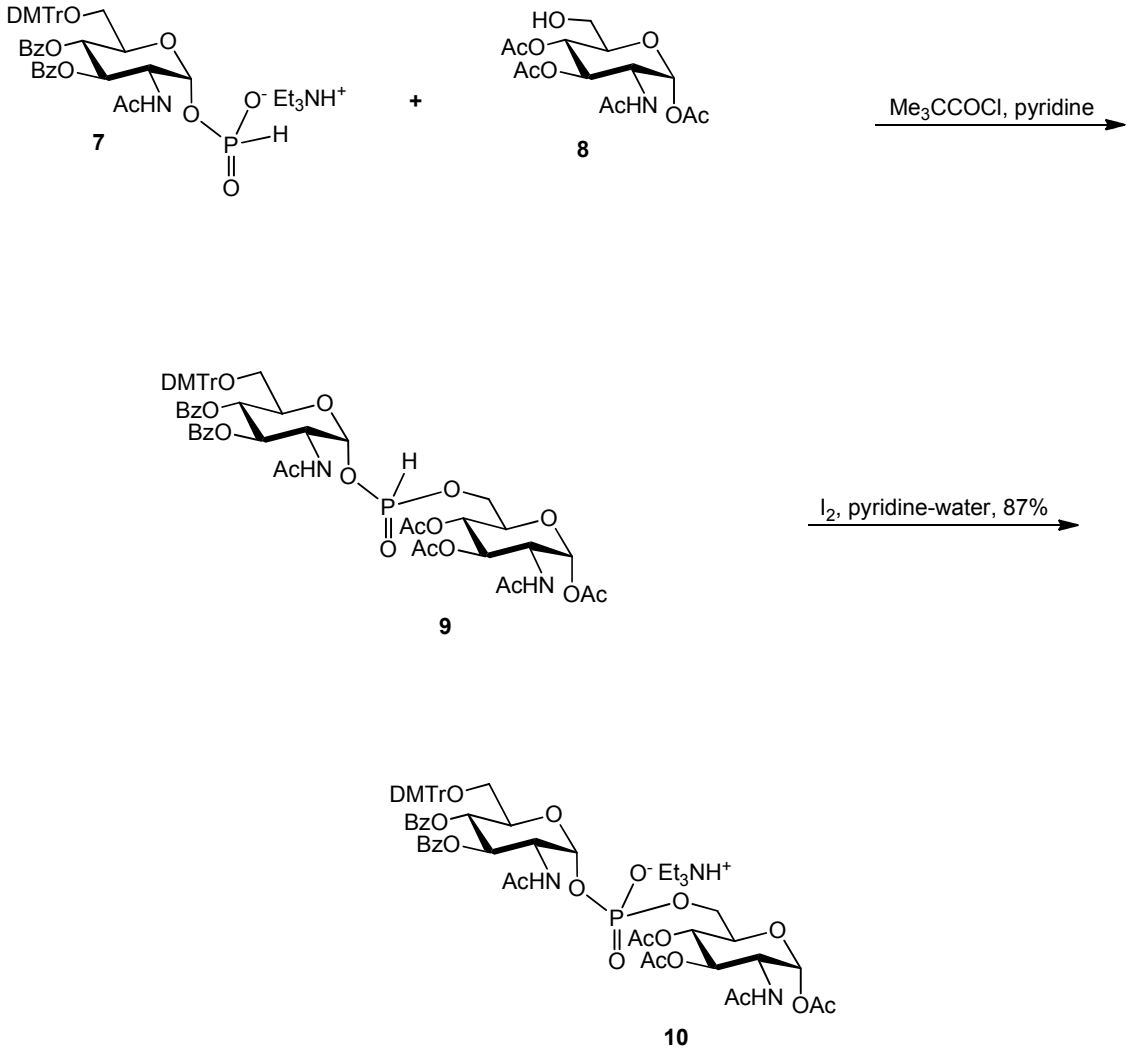
1.2 CHEMICAL INTRODUCTION

1.2.1 The Hydrogenphosphonate (H-phosphonate) approach

The H-phosphonate approach is a highly effective method for the formation of phosphodiester linkages. It was first explored as a synthetic route to oligonucleotides in the 1950s by Alexander Todd's group.[76] The technique was adapted almost thirty years later as a highly efficient method for the synthesis of oligonucleotides (including solid support synthesis) in the mid-eighties.[77,78,79] This technique combined the benefits of excellent yields and a rapid reaction rate requiring only two simple steps in the elongation cycle consisting of protection group removal followed by nucleotide coupling.

The success of this method led to its exploration as a means to synthesise disaccharide phosphates containing intersaccharidic phosphodiester linkages, which are inherently more hydrolytically labile due to a possible formation of a glycosyl cation upon cleavage of the glycosyl phosphate bond. It was first investigated in 1986 with a publication by van Boom and co-workers[80] followed closely by another independent publication by Nikolaev and co-workers in 1987.[81] In the van Boom synthesis a small fragment of the cell-wall polymerof *Staphylococcus lactis* was prepared(see **Scheme 1**). In this synthesis the glycosyl H-phosphonate **7** was combined in pyridine with the N-acetyl-D-glucosamine derivative **8**. Following the addition of the condensing agent pivaloyl chloride the H-phosphonate reacts rapidly with the primary hydroxyl of **9**.After only 10 minutes, analysis with thin layer chromatography (TLC) revealed the

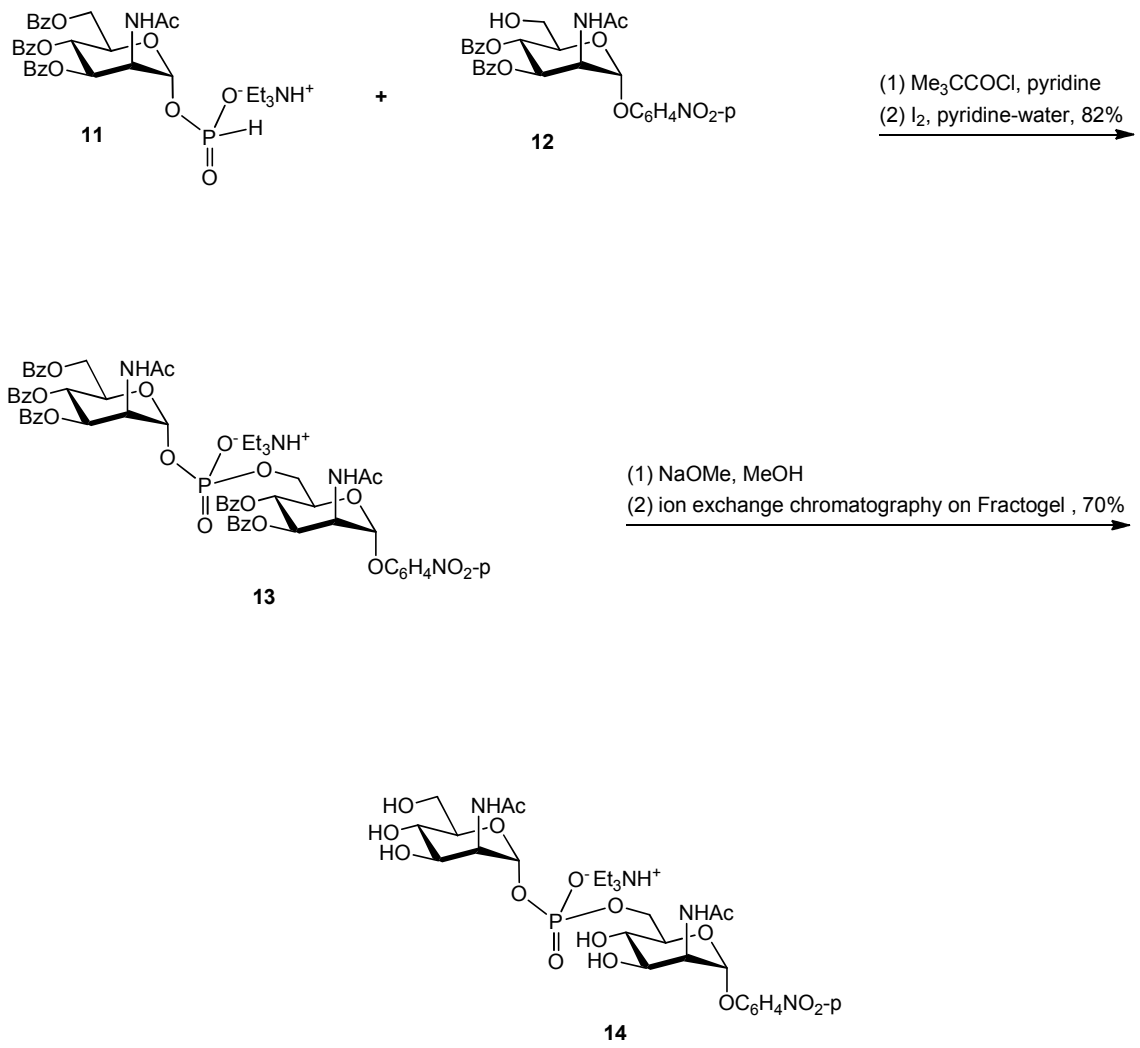
condensation had gone to completion. This was also confirmed with ^{31}P-NMR which revealed only the characteristic signals of an H-phosphonic diester **9** (δ_P; 9.16 and 8.55 ppm, $J_{H,P}$ 737 and 730 Hz). With the condensation complete a solution of iodine in pyridine-water was added to facilitate the *insitu* oxidation of the H-phosphonic diester to form the disaccharide phosphate **10** in 87% yield after purification.



Scheme 1: Initial van Boom glycosyl H-phosphonate condensation.

1.2.2 Synthesis of *Neisseria meningitidis* group Acapsular polysaccharide fragments

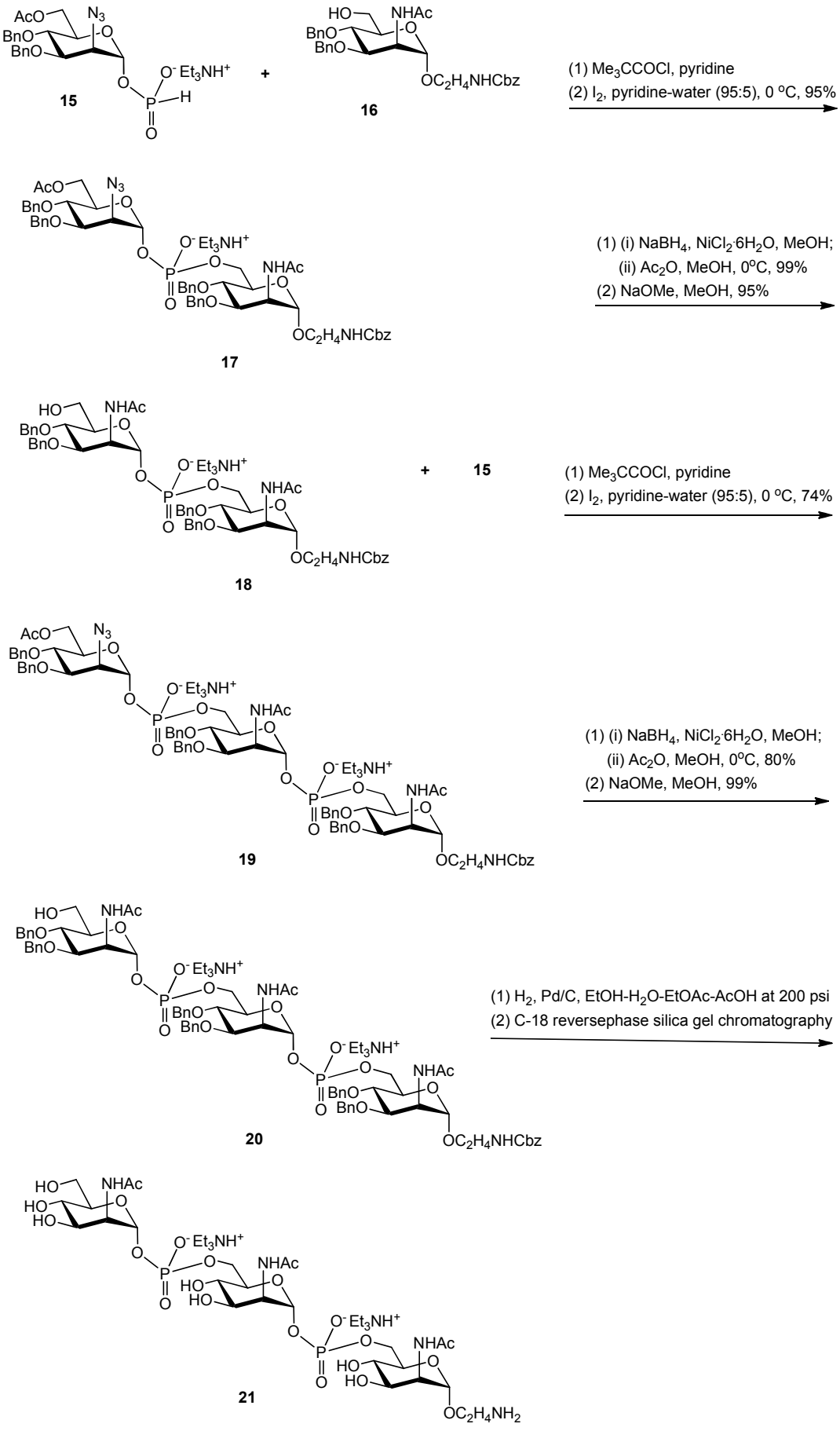
Initial work on the synthesis of fragments of the capsular polysaccharide of *N. meningitidis* serogroup A was carried out in 1993 by Nikolaev and co-workers in Moscow.[82] During this early work they were able to synthesise the disaccharide phosphate **13** through the condensation of the H-phosphonate **11** with the free primary hydroxyl of the N-acetyl-D-mannosamine derivative **12** (see **Scheme 2**). The condensation proceeded through the usual conditions with the addition of the condensing agent pivaloyl chloride in a solution of pyridine. The H-phosphonic diester was then oxidised, again with the addition of iodine solution in pyridine-water, to achieve the protected disaccharide phosphate **13** in 82% yield. After benzoate removal facilitated with sodium methoxidein MeOH,the desired disaccharide phosphate **14** was isolated by ion exchange chromatography in a high yield of 70%.



Scheme 2: First synthesis of *N. meningitidis* serogroup A capsule phosphoglycan fragment.

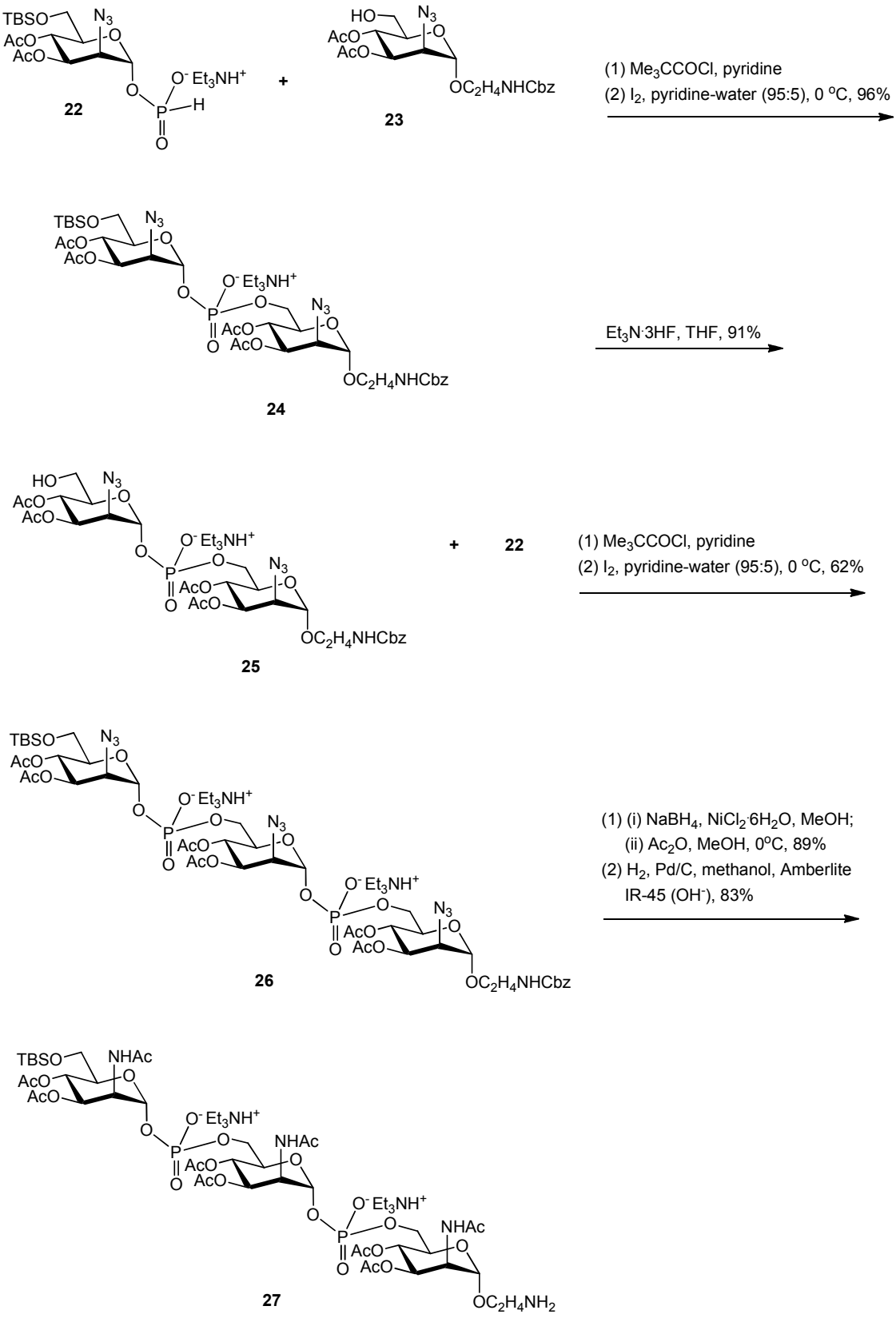
Further progress in the synthesis of fragments of the capsular polysaccharide of *N. meningitidis* serogroup A was achieved by Pozsgay and co-workers as shown in **Scheme 3**.[83] Their strategy employed the 2-azido-2-deoxy-D-mannosyl H-phosphonate derivative **15** and utilised benzyl ethers as permanent protection at the O-3 and O-4 positions while a 6-O-acetyl was used as temporary protection. This was coupled in a condensation with the N-acetyl-D-mannosamine derivative **16** containing aN-benzyloxycarbonyl (Cbz) protected ethanolamine linker at the anomeric position and a free hydroxyl at the C-6 position. Following condensation and oxidation the

disaccharide phosphate **17** was isolated in an excellent yield of 95%.The azide functionality of the disaccharide phosphate **17** then underwent reduction using sodium borohydride and nickel chloride to furnish a free amine, whichwas then acetylated *in situ* with the addition of acetic anhydride in methanol. The temporary protection of the O-6 position was removed using Zemplen conditions to give derivative **18,** which was then ready for further chain elongation. The monohydroxyl derivative **18** was then combined with the H-phosphonate derivative **15** in pyridine and condensation was again initiated with pivaloyl chloride.Following oxidation the trisaccharide diphosphate **19** was isolatedin a good yield of 74%. Although a good yield was achieved there is still a noticeable decrease in efficiency when the second intersaccharidic phosphate is introduced. This is in contrast with oligonucleotide synthesis where every condensation gives an excellent yield even after several chain elongations. The azido group of **19** was then reduced and N-acetylated as above before the removal of the O-acetate, again using Zemplen conditions, to give derivative **20**.Finally the trisaccharide diphosphate **20** was subjected to catalytic hydrogenolysis in order to remove the O-benzyl and N-benzyloxycarbonyl protection to afford the phosphoglycan **21** containing a small linker region with a free amine functionality giving the ability to couple to a carrier protein.



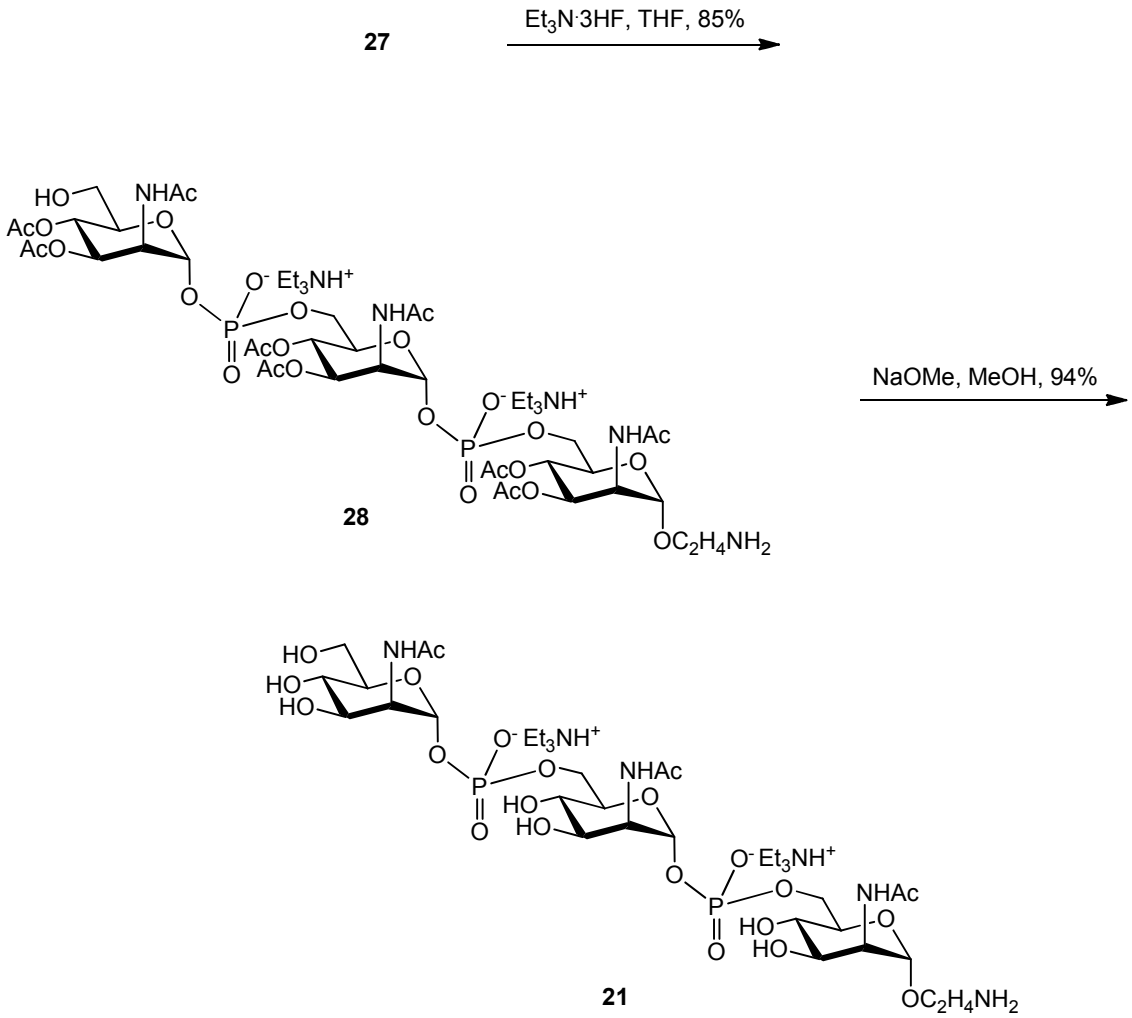
Scheme 3: Pozsgay and co-workers synthesis.

Another synthetic route tocapsular polysaccharide fragments, including the same phosphoglycan **21** using a different synthetic strategy was carried out by the Oscarson group in Stockholm.[84] Their method used a different protecting group arrangement with the use of O-acetates as permanent protecting groups and a *tert*-butyldimethylsilyl (TBS) as a temporary O-6 protection (see **Scheme 4**).In this synthesis the authors also opted to use azide functionalities throughout the process with reduction and N-acetylation as a final step. Using this arrangement the authors were able to perform the first condensation of the 2-azido-2-deoxy-D-mannosyl H-phosphonate derivative **22** and the monohydroxyl containing azidomannose derivative **23** to give the disaccharide phosphate **24**with an excellent yield of 96% after oxidation. The temporary silyl protection was then removed with a solution of triethylamine tri(hydrogen fluoride)to afford the disaccharide phosphate **25**, again with an excellent yield of 91%, and with the free hydroxyl required at the terminal O-6 position ready for another step elongation. The introduction of the second intersaccharidic phosphate was achieved with the condensation of the disaccharide phosphate **25** and the same H-phosphonate **22** and, following oxidation, the trisaccharide diphosphate **26** was isolated in 62% yield. This second condensation also showed a similar drop in yield as was seen in the Pozsgay synthesis with the addition of a second intersaccharidic phosphate. The azide functionalities were then reduced to amines using sodium borohydride and nickel chloride before performing N-acetylation *in situ*.



Scheme 4: Oscarson group synthesis of trisaccharide diphosphate.

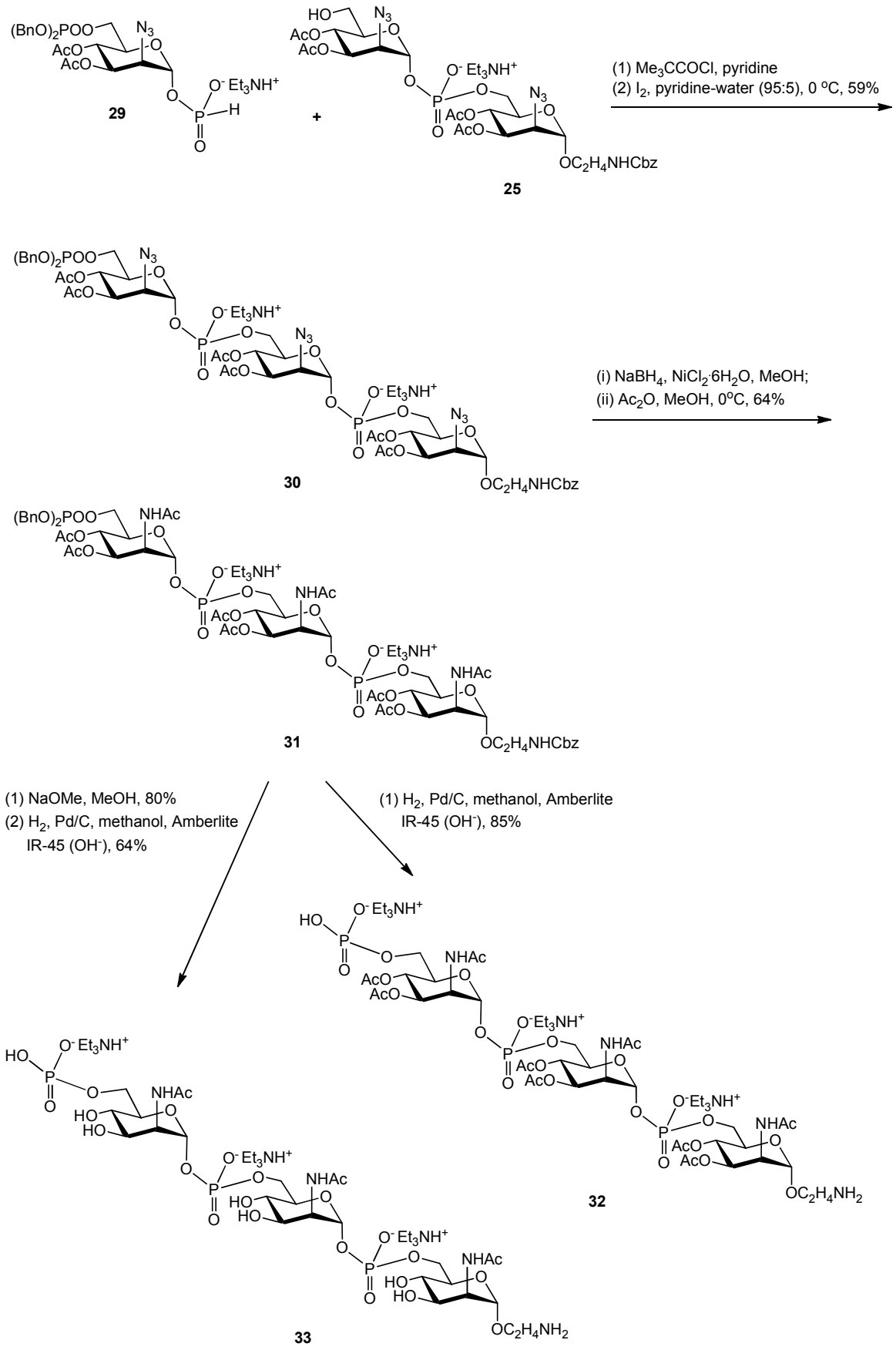
The N-benzyloxycarbonyl protecting group was then removed using hydrogenolysis to give the trisaccharide diphosphate **27** in a good yield of 83%. Finally the TBS protection was removed to give trisaccharide diphosphate **28** in a good yield of85% (see **Scheme 5**).



Scheme 5: Oscarson group trisaccharide diphosphate deprotection.

With acetates at both the O-3 and O-4 position the trisaccharide diphosphate **28** better resembles the native polysaccharide, which only contains one acetate, usually at the O-3 position but occasionally the O-4 position. These acetates were also cleaved off using sodium methoxide to furnish the trisaccharide diphosphate **21** replicating the fragment synthesised by the Pozsgay group.

The Stockholm researchers also developed a strategy allowing the synthesis of 6''-O-phosphorylated phosphoglycans (see **Scheme 6**). They achieved this by producing the 6-O-phosphorylated H-phosphonate derivative **29** and using this in the condensation (in standard conditions) with the previously synthesised monohydroxyl derivative **25**. Following oxidation the trisaccharide triphosphate derivative **30** was achieved in a similar yield for second phosphate introduction of 59%. The azides of **30** werethen reduced to amines again using sodium borohydride and nickel chloride before the addition of acetic anhydride to facilitate N-acetylation *in situ* to furnish the trisaccharide triphosphate derivative **31**.Finally, the N-benzyloxycarbonyl protection was removed from the ethanolamine linker by hydrogenolysis to achieve the desired trisaccharide triphosphate **32,** which again contained acetates at the O-3 and O-4 position, in good yield. To achieve yet another derivative the acetates were first removed from derivative **31** with sodium methoxide before removal of the N-benzyloxycarbonyl protecting group using hydrogenolysis to give the trisaccharide triphosphate **33**again in good yield.



Scheme 6: Oscarson group synthesis of trisaccharide triphosphate compounds.

1.2.3 Synthetic glycoconjugate vaccines for *Haemophilus influenza* type b

An alternative route for the production of glycoconjugate vaccines (see section **1.1.6**) is to chemically synthesise fragments of the capsular polysaccharide. One of the issues with naturally derived carbohydrate polymers is that they are heterogeneous mixtures that may include contaminants and unwanted impurities. Through the use of synthetic carbohydrate structures, which can be chemically produced as single compounds, batch to batch variability can be eliminated. For example, a fully synthetic hexasaccharide conjugated to keyhole limpet hemocyanin (KLH) has been shown to be an effective vaccine against prostate cancer.[85] This methodology has also been used by Verez-Bencomo et al. with great success in manufacturing a fully synthetic vaccine against *Haemophilus influenza* type b (Hib).[86]Synthetic preparation of the glycan part of the glycoconjugate vaccines also allows for chemical modifications to the structure that may prove difficult or impossible to perform on the naturally derived material.

With regard to the Hib vaccine the group had to first synthesise a fragment of the capsular polysaccharide of Hib, which is composed of ribosylribitol phosphate repeating units (see **Figure 7**). One alteration to this fragment was the addition of a linker region to the primary unit, which would allow the coupling of the fragment to a carrier protein.

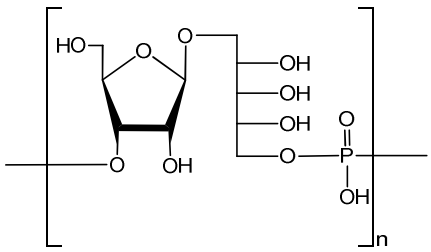
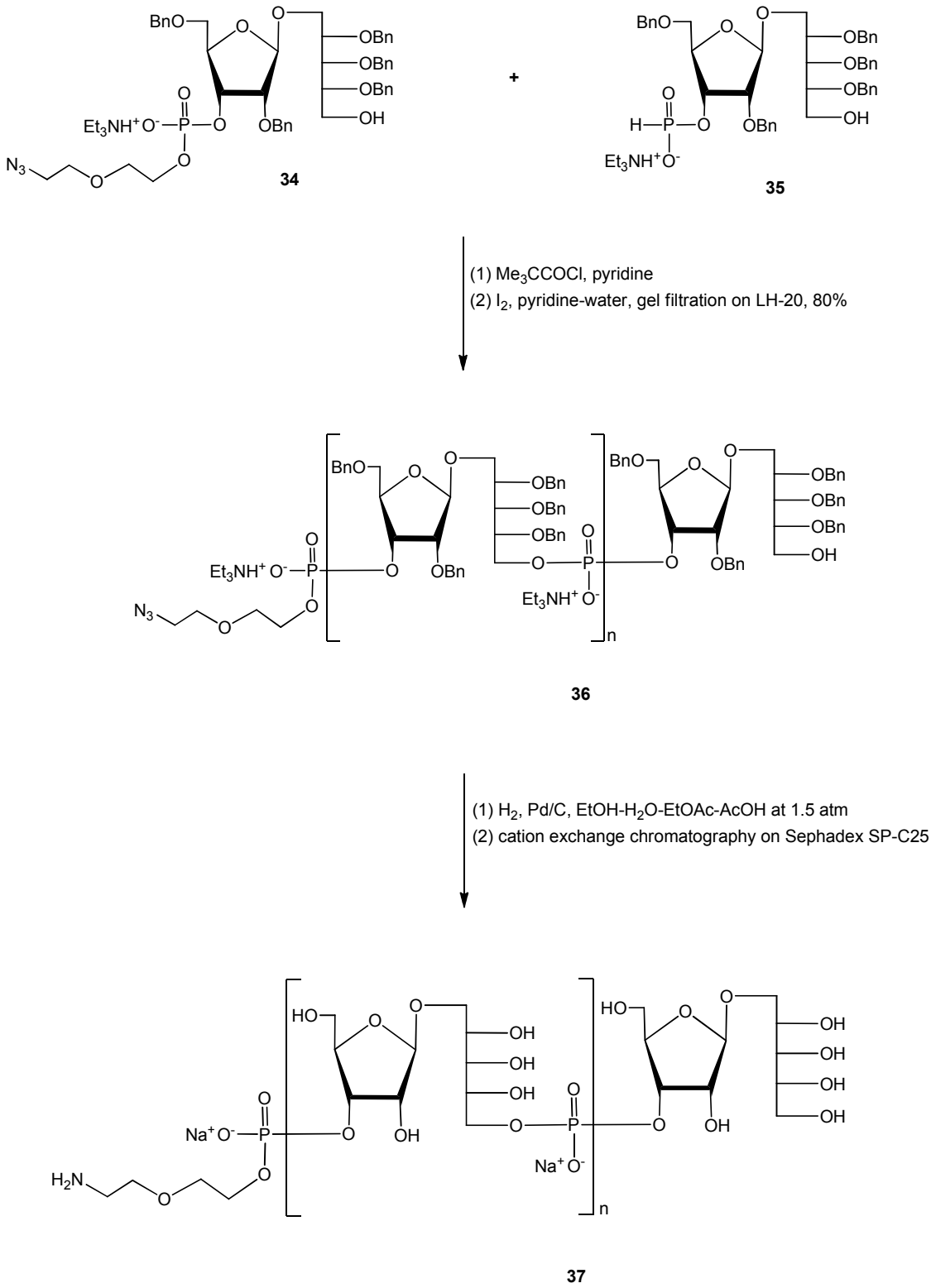


Figure 7: Repeat unit of Hib capsular polysaccharide.

The aim of the researchers was to develop a synthetic methodology that would also allow the large scale good manufacturing practice (GMP) production of the fragments. With this in mind they set out to develop a strategy that would minimise the number of reaction and chromatography purification steps in order to simplify production. Their

approach utilised the reliable and efficient H-phosphonate chemistry (see **Scheme 7**) in their synthesis for the formation of the phosphodiester linkages.[87]



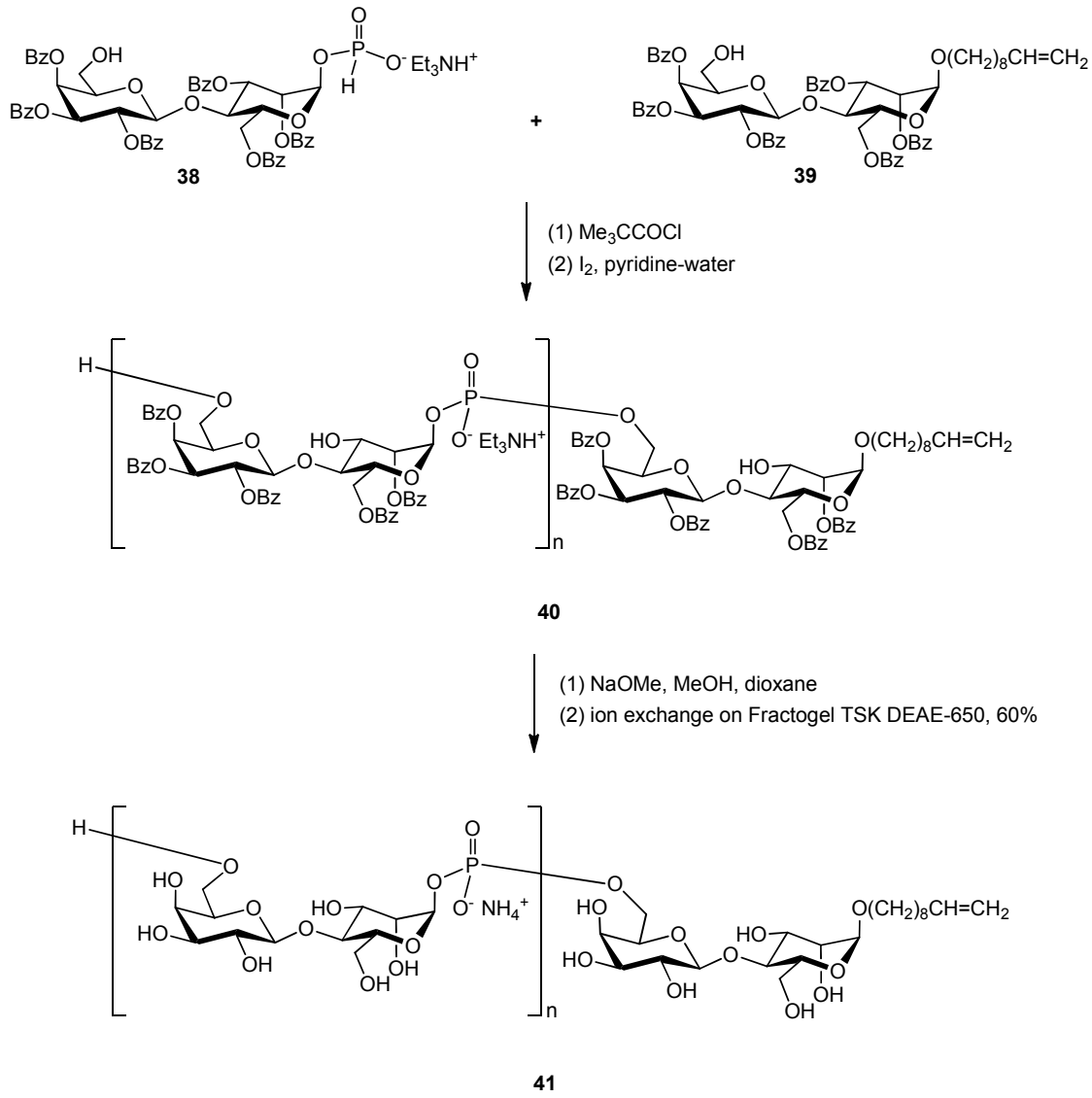
Scheme 7: Route to Cuban synthetic vaccine.

This has proven to be the method of choice for the efficient construction of various phosphodiester linkages which are characteristic of natural phosphoglycans.[88] Additionally, by the use of the H-phosphonic synthon **35**,which also has a free hydroxyl group the reaction proceeded through a co-polycondensation process after the addition of the condensing reagent pivaloyl chloride.[87]Following the co-polycondensation of the H-phosphonate **35** with the linker-phosphate containing monohydroxyl unit **34** the H-phosphonic diesters formed were oxidised readily to phosphodiesters with the addition of iodine in pyridine-water to furnish the protected phosphoglycan**36**. This allowed the production of long chain oligomers in a one-pot synthesis and enabled the researchers to reproducibly synthesise oligomers with an average size of 8 repeating units with an impressively high yield of 80% after being purified by size exclusion chromatography. After removal of the benzyl protecting groups and reduction of the azide with hydrogenolysis the desired oligomeric phosphoglycan**37** was isolated with a primary amine functionality capable of conjugation to a carrier protein. The beauty and straightforwardness of this approach, which employed only five steps, allowed the whole process to be efficiently scaled up and proceed under Good Manufacturing Process (GMP) to provide 100 g per batch.The results of a clinical trial demonstrated that the synthetic glycovaccine is as effective as its natural counterpart. The vaccine was registered in Cuba in 2003 and is now part of the national immunization program.

1.2.4 Synthetic neoglycoconjugates of *Leishmania* lipophosphoglycan

Another nice exampleof using synthesised phosphoglycans containing a linker region to produce neoglycoconjugates as a potential vaccine was demonstrated in the synthesis of neoglycoconjugates of*Leishmania* lipophosphoglycan.

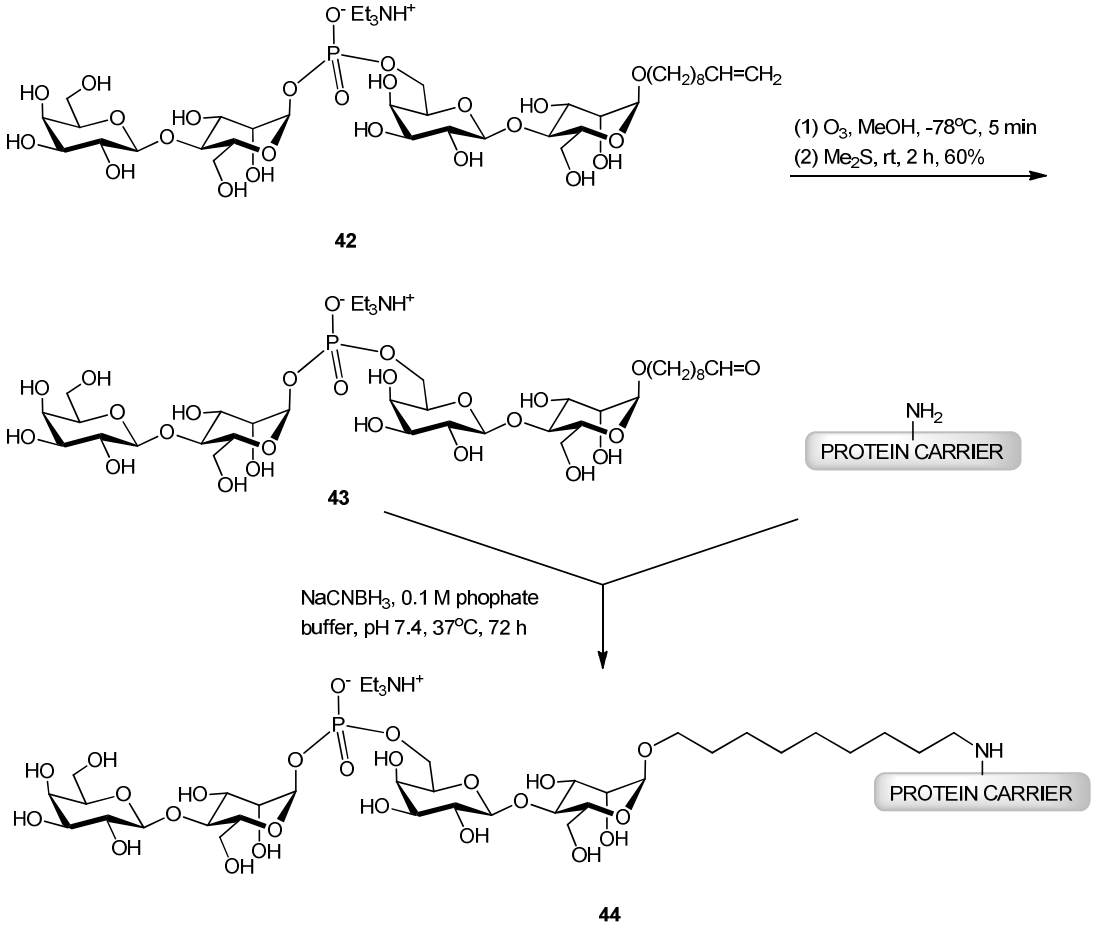
A characteristic part of the *Leishmania* parasite is a complex glycoconjugate called the lipophosphoglycan (LPG).[89,90] The most distinctive part of the *Leishmania* LPG is their variable phosphoglycan domain consisting of a β-D-Gal-(1→4)-α-Man1→OPO_3phosphosaccharide repeat. The synthesis of which is challenging due to the phosphoglycans being hydrolytically unstable under reaction conditions and further complicated by the need for the correct stereochemistry at the C1 position of the phosphodiester linkage. The first successful synthesis of the *Leishmania* phosphoglycans was achieved by the Dundee University group by again exploiting the H-phosphonate approach.[91,92] All the phosphoglycans prepared contained a dec-9-enyl aglycone moiety which was purposely designed to both aid in the study of *Leishmania* biosynthetic enzymes and, after modification, allow for the preparation of neoglycoconjugates.[93] An example of such a preparation is shown in **Scheme 8**. This approach employed the co-polycondensation technique by coupling the primary hydroxyl unit **39** with the monohydroxyl H-phosphonate derivative**38**. The H-phosphonate **38** has a free primary hydroxyl group at the C-6 position, which allows for further condensation of the remaining H-phosphonate, which was present in a relatively high concentration of 1 M. Historically, this was the first phosphoglycan synthesised (in 1995) using the H-phosphonate polycondensation technique,[87]and which was later followed (in 2004) by the Cuban researchers(see section **1.2.3**).[86] _ENREF_76



Scheme 8: Synthesis of *Leishmania* LPG fragment.

The phosphoglycan **40** was then debenzoylated with sodium methoxide and following purification through ion-exchange chromatography on Fractogel TSK DEAE-650 (HCO_3^-) the desired phosphoglycan **41** (n ~ 6)wasisolated in a yield of 60%.

Phosphoglycans containing the dec-9-enyl moiety could then be used for the preparation of neoglycoconjugates via the oxidation of the double bond through the mild conditions of ozonolysis to afford an aldehyde moiety. Now with the linker region terminating in an aldehyde functionality, it was shown that the phosphoglycans could be coupled to a carrier protein through reductive amination technique with a free amino group of the carrier protein, an example of which is shown in **Scheme 9**.[94,95,96]



Scheme 9: Synthesis of neoglycoconjugatesby Dundee University group.

This procedure was repeated with an array of synthesised LPG structures[91,92,97,98,99,100]to yield a range of neoglycoconjugates of progressively larger phosphoglycans and branching phosphoglycans shown in **Figure 8**.

Although structurally unrelated to the capsular polysaccharide of Men-A the above synthesis does demonstrate two key points. The first being that the H-phosphonate approach is the method of choice for the synthesis of natural phosphoglycans and secondly that the dec-9-enyl linker proved efficient and reliable in the preparation of neoglycoconjugates from structure containing intersaccharidic phosphate linkages.

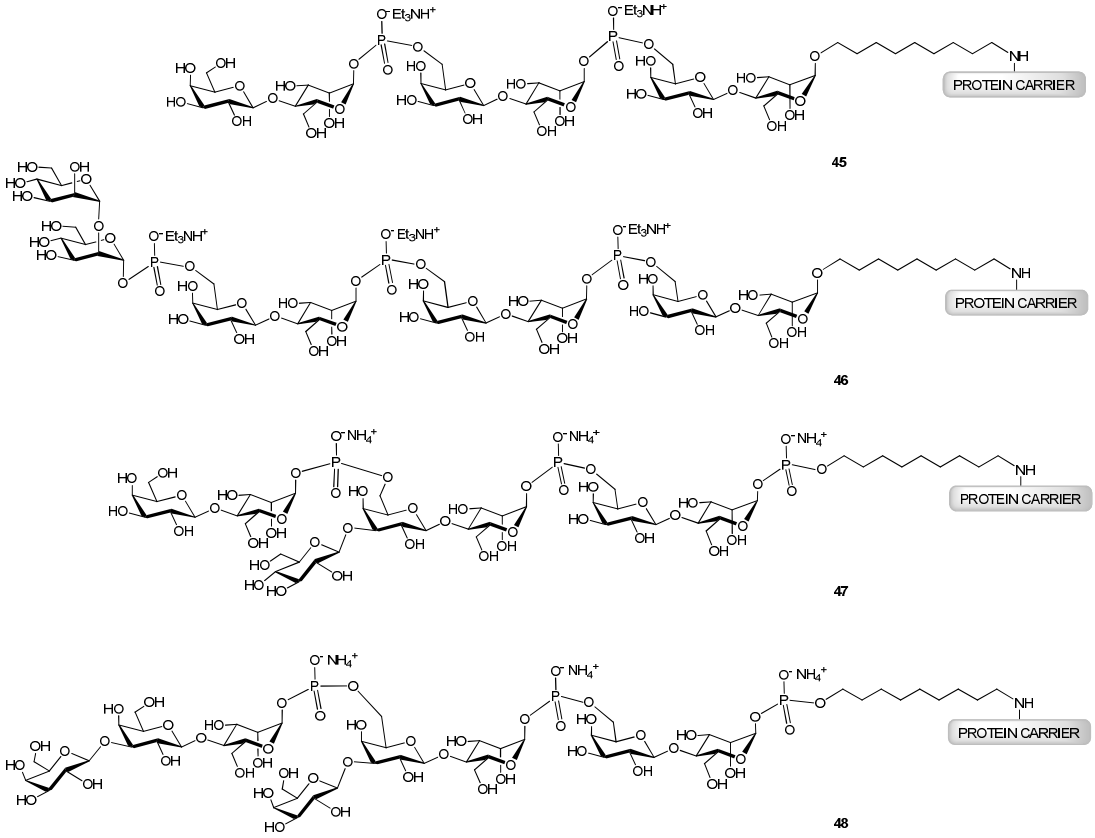
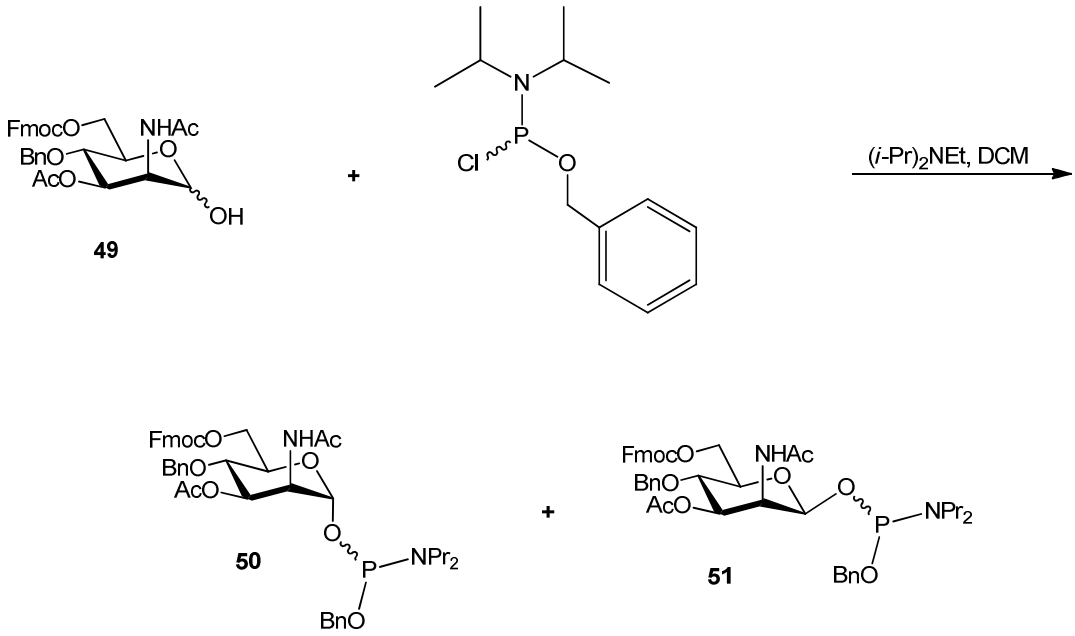


Figure 8: Neoglycoconjugates of synthetic *Leishmania* phosphoglycans.

1.2.5 Phosphoramidite synthons

An alternative method to the formation of phosphodiester bonds is the phosphoramidite approach. This has been used with great success in the chemical synthesis of oligonucleotides for many years.[101,102] The preparation of suitably protected 2-acetamido-3-O-acetyl-2-deoxy-D-mannose phosphoramidite derivatives was shown by Vann and Freese in 1996 (see **Scheme 10**).[103]Unfortunately the paper only describes the synthesis of synthons **47** and **48**and does not mention any attempts to use them in the formation of a phosphodiester.



Scheme 10: Phosphoramidite synthons related to *N. meningitidis* serogroup A capsulephosphoglycan.

CHAPTER 2

2. RESULTS AND DISCUSSION

2.1 AIMS OF THE PROJECT

2.1.1 Synthetic targets

As mentioned earlier in section **1.1.4**, the capsular polysaccharide(CPS) of *Neisseria meningitidis*serogroup A forms a dense protective layer of(1-6)-linked poly(2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate)around the cell and provides protection from the surrounding environment (**Figure 5**).Although successful, previous work on the synthesis of the CPS fragments stopped after the introduction of the second intersaccharidic phosphate and the fragments produced had either no acetate groups or acetates at both the O-3 and O-4 positions. The importance of this O-acetylation to the immunogenicity of the capsular fragments has been shown by Berry and co-workers to be of great significance.[34] In their studies it was shown that antibodies against the native CPS of *Neisseria meningitides*serogroup A had a reduced ability to bind capsular fragments that had been de-O-acetylated. Moreover the immunogenicity of de-O-acetylated CPS fragments was markedly reduced, even when conjugated to a tetanus toxoid protein.[34]

Therefore, to better replicate the native CPS structure, a synthetic strategy resulting in only 3-O acetates was proposed with the additional aim of producing larger fragments with more than two intersaccharidic phosphates (see **Figure 8**).

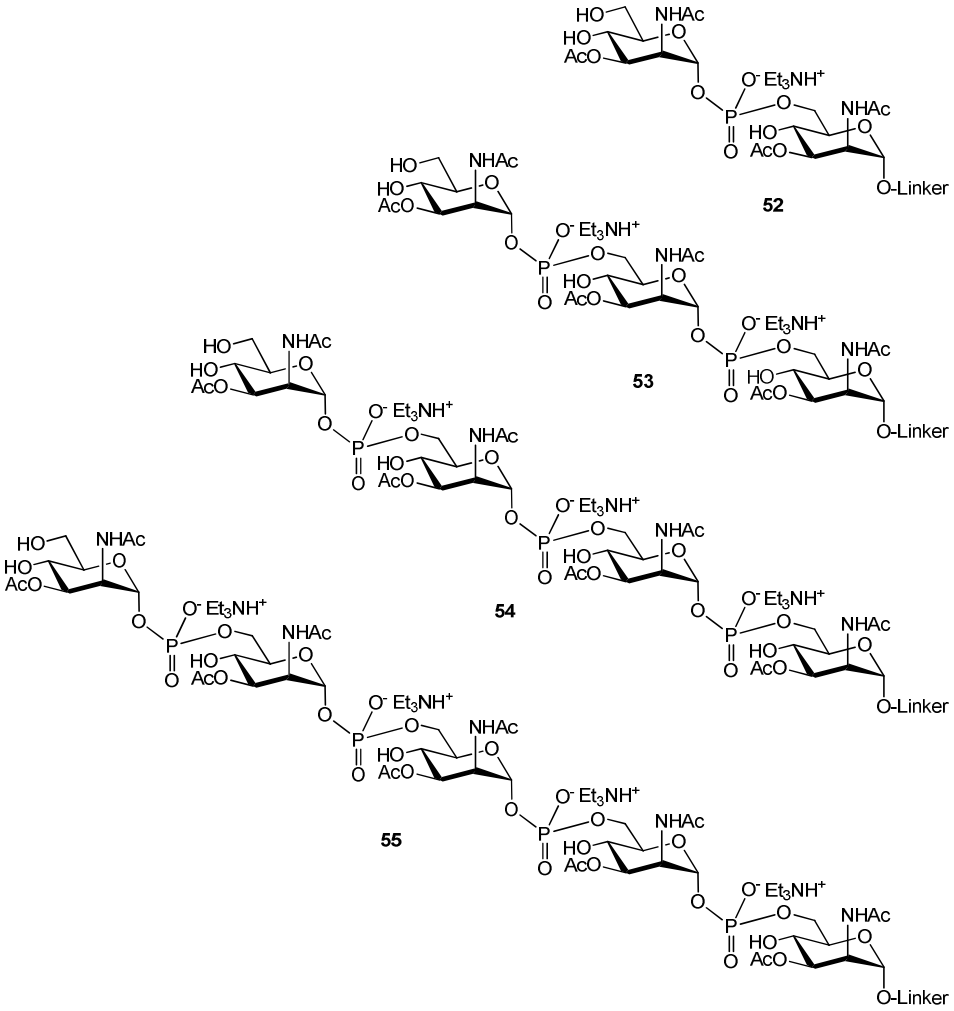


Figure 9: Proposed synthetic target phosphoglycans.

As shown above, the original aim of this project was to synthesise fragments ranging in size from 1 to 4 intersaccharidic phosphates. All of the synthetic targets **52-55** also contain an integrated linker at the reducing end of the phosphoglycan chain allowing for conjugation of the capsular fragments to protein.

2.1.2 Stability of the glycosyl phosphate bond in N-acetylmannosamine phosphate derivatives

The chemical composition of this capsule however provides a particular challenge to any chemical synthesis due to the intersaccharidic phosphodiester linkage which is inherently unstable under reaction conditions. This is further destabilised by the

presence of the N-acetyl moiety of carbon 2 which can participate in the cleavage of the phosphodiester bond (see **Figure 9**).

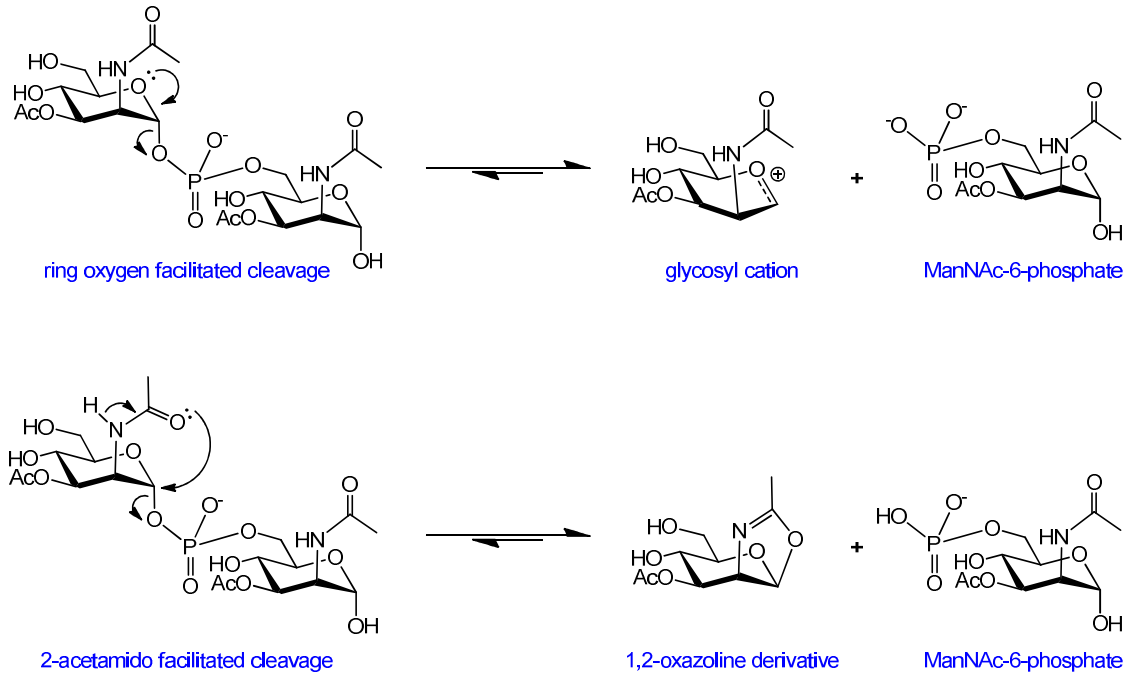


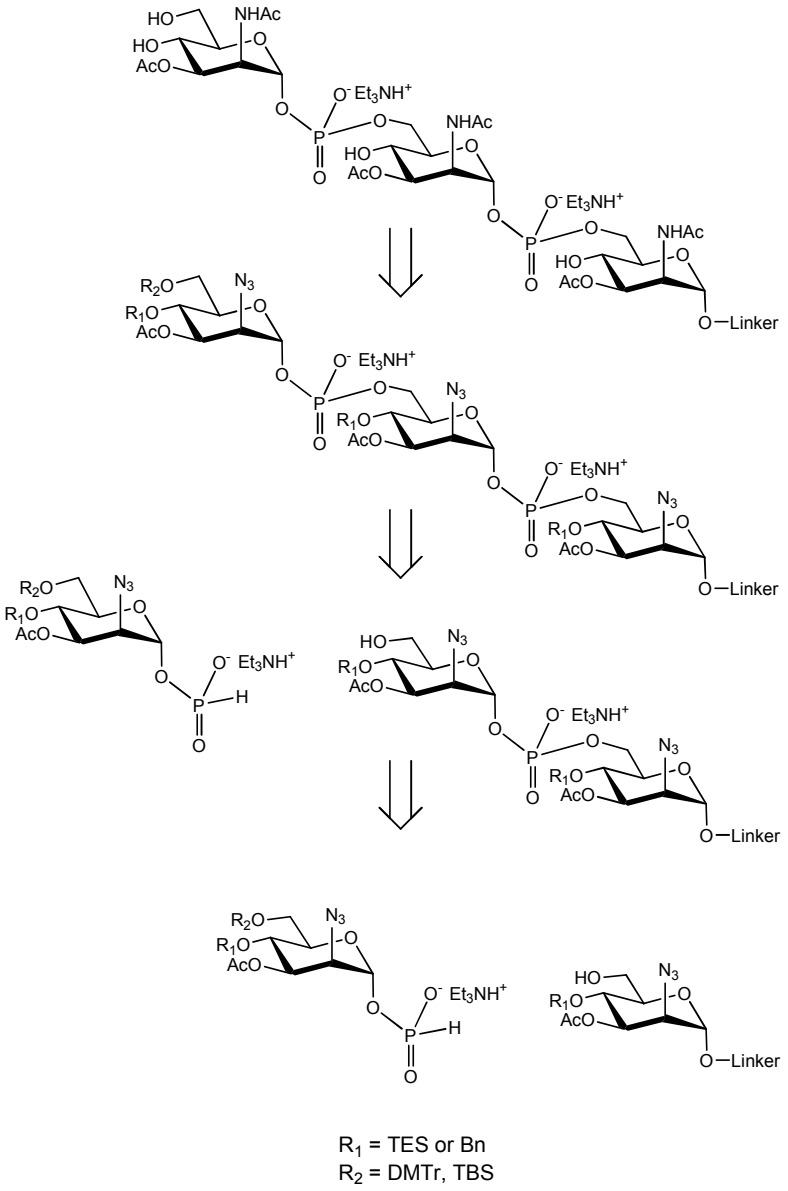
Figure 10: Instability of N-acetylmannosamine phosphate linkage.

Like all intersaccharidic phosphodiester bonds, under reaction conditions, the ring oxygen of the carbohydrate can donate a pair of electrons to facilitate cleavage of the glycosyl phosphate bond. This is energetically favourable due to the resulting ManNAc 6-phosphate being a good leaving group while the remaining glycosyl cation is also relatively stable entity. The most instability however comes from the oxygen of the acetamido group which in this structure sits in the axial configuration of carbon 2. This leads to the formation of a stable 1,2-oxazoline derivative and again ManNAc 6-phosphate as the leaving group.

2.2 RETROSYNTHETIC ANALYSIS

2.2.1 Retrosynthetic analysis of CPS synthesis

In order to reduce the instability of the glycosyl phosphate potentiated by the acetamido group, during the synthesis an azido functionality was used at the 2 position. This proved successful during the synthesis of capsule fragments mentioned earlier (**Scheme 3** and **Scheme 4**)by the Pozgay and Oscarson groups respectively. Although successful the synthesis stopped after the introduction of the second intersaccharidic phosphate and the fragments produced had either no acetates or acetates at both the O-3 and O-4 positions. To better replicate the native CPS structure a synthetic strategy resulting in only 3-O acetates was proposed with the additional aim of producing larger fragments with more than two intersaccharidic phosphates (see **Scheme 11**).



Scheme 11: Retrosynthetic analysis of capsule fragments.

Another important feature required for a potential vaccine is the ability to conjugate to a carrier protein. In this case it was thought that a 9-decen-1-yl moiety attached to the primary saccharide unit would make a good candidate. It has previously been used successfully by the Nikolaev group in the development of a potential anti-*Leismania* vaccine.[94,96] Once installed the 9-decen-1-yl group is stable under a range of reaction conditions and the double bond can be readily oxidized to form an aldehyde functionality using ozonolysis. This aldehyde moiety can then facilitate conjugation through reductive amination.

The elongation of the fragments would proceed through the condensation of an orthogonally protected monomeric 2-azidomannose H-phosphonate derivative with the primary 2-azidomannose residue complete with decenyl spacer and the free hydroxyl required at the C-6 position. Following condensation of the H-phosphonate and subsequent oxidation to the phosphodiester the temporary protection at the O-6' position can be selectively removed to provide the hydroxyl required for further chain elongation.

2.2.2 Orthogonally protected monosaccharide synthons

To explorethe optimal combination for the permanent O-4 protection and the temporaryO-6 protection four different orthogonally protected monosaccharides were designed (see **Figure 9**).

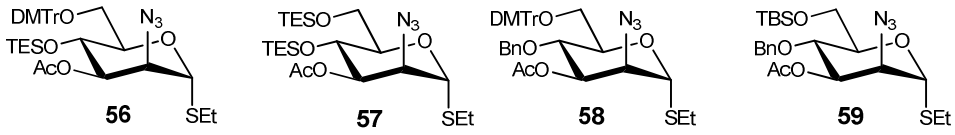


Figure 11: Orthogonally protected monosaccharide synthons.

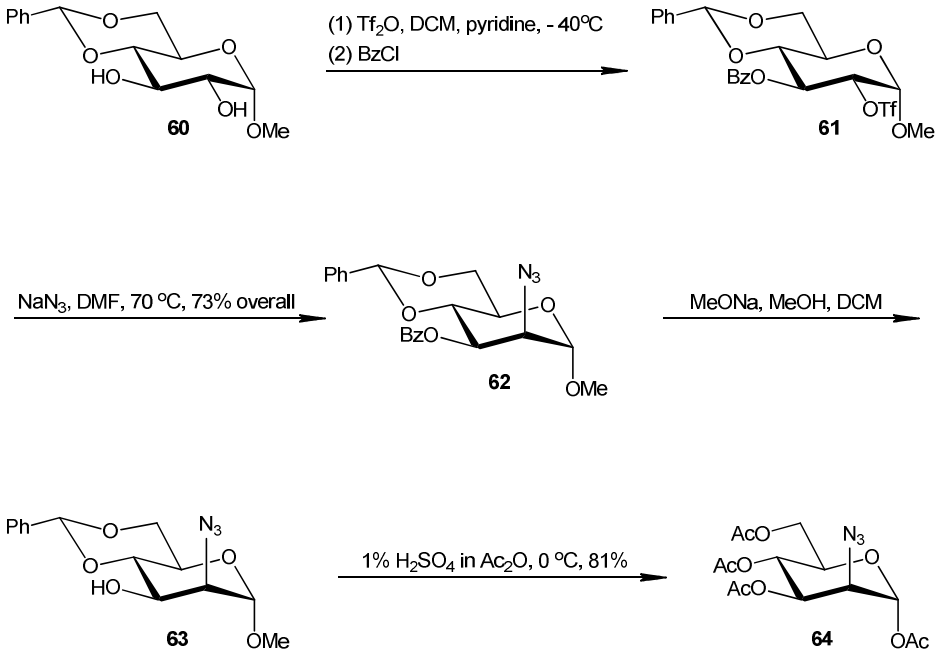
Each compound comprises a selectively cleavable ether at the O-6 position to facilitate chain elongation and a permanent silyl or benzyl protecting group at the O-4 position, both of which can be removed while leaving the 3-O acetate intact. To prevent the aforementioned instability arising from the 2-acetamido group in the native ManNAc structure the N-acetyl group was 'masked' as a 2-azido group. This pseudo-protecting group can be readily reduced, followed by N-acetylation,in the final stages of the synthesis. The designed synthons also contain an ethanethiol group at the anomeric

position, which through hydrolysis can form the hemiacetal required for the formation of the desired H-phosphonates. Aside from being readily hydrolysed the thioglycosides also make efficient glycosyl donors, a property required for the glycosylation between the primary saccharide unit and the desired linker.

2.3 SYNTHESIS OF MONOSACCHARIDE SYNTHONS

2.3.1 Synthesis of 1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose

A common precursor to all of the thioglycosides shown in**Figure 9**is 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose**64** (see **Scheme 12**). The preparation of this tetra-acetate derivative found in literature was either low yielding or involved a complicated anhydrous work-up.[104,105]



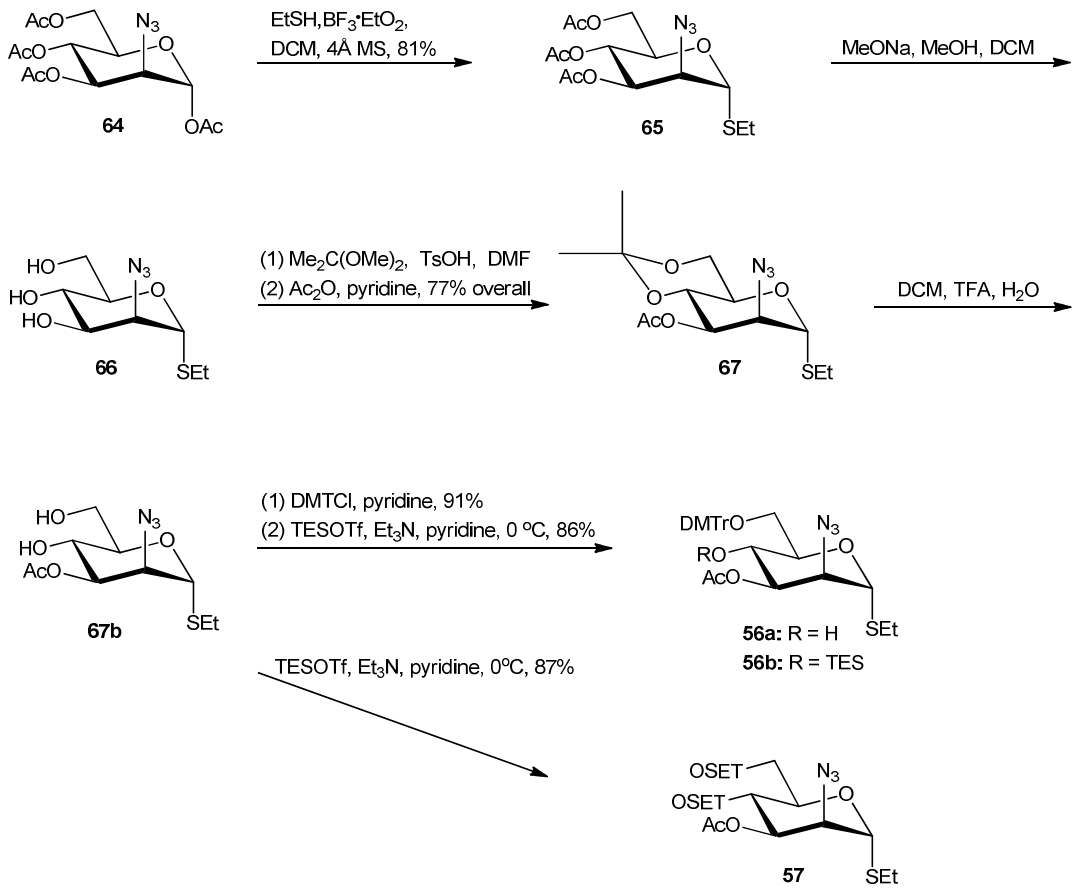
Scheme 12: Synthesis of 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose.

In order to avoid these complications a different synthetic route was developed. In this preparation, first the 4,6-benzylidene-protected methyl glucoside**60** was selectively triflylated at the O-2 position using trifluoromethanesulfonic anhydride at -40ºC. The progress was monitored with TLC until no trace of the diol **60**remained. The O-3 position was then benzoylated with the addition of benzoyl chloride while the reaction was allowed to warm to room temperature. After work up the cude triflate derivative **61** was obtained. Derivative **61** could then be used without the need for any purification in the triflate displacement with sodium azide. This resulted in an inversion of the configuration, which afforded the azido-mannose derivative **62**in a good yield of 96%.[106] The next step was to convert to the peracetylated 2-azido-2-deoxy-manose. This was done by first removing the benzoyl ester at the O-3 position with transesterification using sodium methoxide in a solution of methanol and dichloromethane. After debenzoylation compound **63** was subjected to acetolysis in the presence of 1% (v/v) sulphuric acid in acetic anhydride, facilitating cleavage of both the anomeric O-methyl and the benzylidene acetal while fully acetylating all positions. This gave the target tetra-acetate **64** in a very respectable yield of 81%.

2.3.2 Synthesis of the thioglycoside synthons 56-59

The next stage in the synthesis required theinstallation of a thiol functionality at the anomeric position (see **Scheme 13**). This was achieved by reactingthe tetra acetate **64** with ethanethiol in the presence of $BF_3{\cdot}EtO_2$ and 4Å molecular sieves.[107] Once full conversion was observed by TLC the reaction was quenched with the addition of triethylamine and the reaction mixture applied directly onto a silica column. This was then flushed with toluene which served to both remove the remaining ethanethiol and additionally filter off the molecular sieves. The flushed toluene could then be safely

discarded with the organic waste eliminating the need for an aqueous workup. The thioglycoside **65**was then acquired in 81% yield by eluting from the column with a 30% solution of ethyl acetate in toluene.



Scheme 13: Synthesis of monosaccharide synthons **56** and **57**.

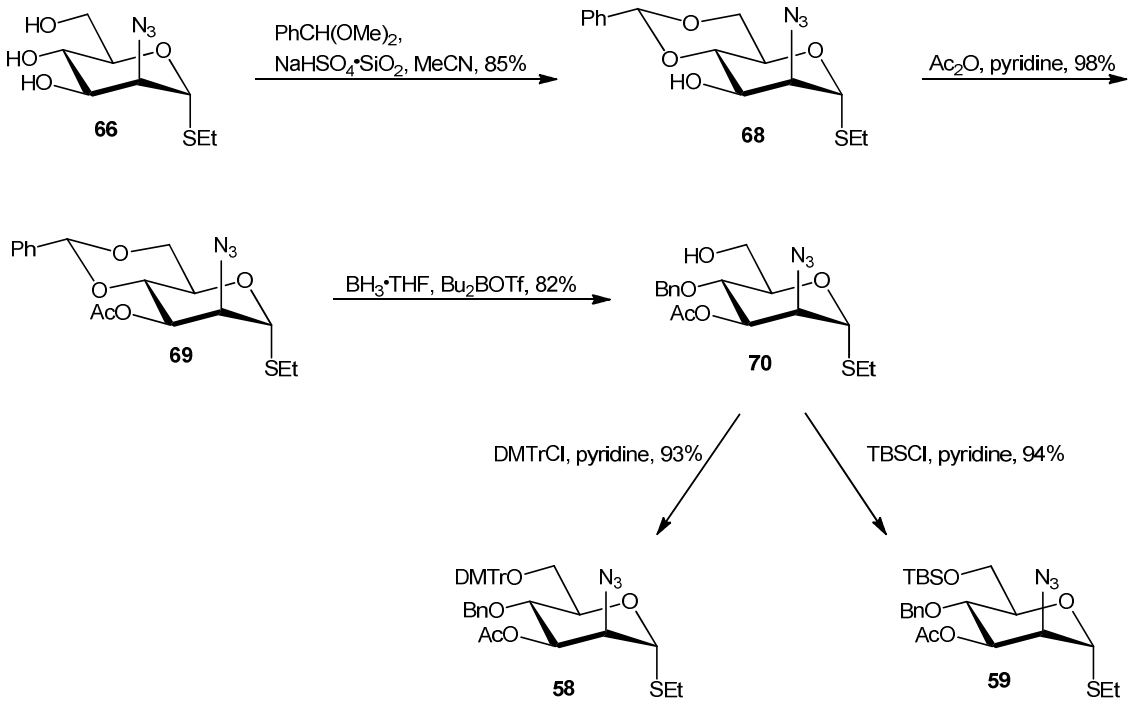
Following successful transformation to the thioglycoside the remaining acetate groups had to be cleaved to allow for further protection group manipulation. This was accomplished again using the standard conditions of sodium methoxide and methanol in a solution of dichloromethane. The solution was then de-ionised with the addition of DOWEX 50 (H^+) resin and stripped of solvent to leave the triol **66**. In order to selectively protect the 4,6-hydroxyls an isopropylidene ketal was formed. This was

installed using 2,2-dimethoxypropane with *p*-toluenesulphonic acid as a catalyst,while monitoring progress by TLC. Once all of the triol had been consumed the remaining O-3 position was acetylated by adding acetic anhydride and pyridine to the reaction. This gave the required compound **67** in a respectable 77% yield. The ketal protection introduced in the previous step was then cleaved by hydrolysis in the presence of trifluoroacetic acid (TFA) to release the 4,6-diol. Selective protection of the C-6 hydroxyl was then achieved by exploiting the increased reactivity of the primary hydroxyl in addition to the sterically hindered nature of the reagent dimethoxytrityl chloride (DMTrCl). Once complete the remaining O-4 position was then silylated with an injection of triethylsilyl trifluoromethanesulfonate (TESOTf) to furnish synthon**56**.

The synthesis of synthon **57** proceeded in a similar manner with the cleavage of the ketal from **67**again to give the 4,6-diol. This was then reacted with a slight excess (2.5 eq) of TESOTf in a solution of pyridine to give an 87% yield of **57**.

For the preparation of synthons **58** and **59** a slightly different approach was taken. This decision was taken to avoid the basic conditions used in the standard benzylation reaction which potentially could cause partial acetyl cleavage or migration. The triol **66** was first protected, again at C-4 and C-6 hydroxyls, using a benzylidene acetal. This was achieved with benzaldehyde dimethylacetal and the heterogeneous catalyst $NaHSO_4$•SiO_2 to yield **68** in 85% yield.[108] The remaining hydroxyl at the C-3 was then acetylated, again using acetic anhydride in pyridine to give **69** in quantitative yield. The six membered ring of the benzylidene acetal could then be selectively opened to release the C-6 hydroxyl and following reduction provide the required benzyl protection at the O-4 position. This reductive cleavage was performed with dibutylboron triflate and borane in THF.[109]Thesteric hindrance around the O-4 position and the bulky nature of the Lewis acid dibutylboron triflate ensured that only the more available O-6 bond was cleaved.Subsequent reduction with borane gives derivative **70**in

82% yield. The free primary hydroxyl group was then protected without difficulty using DMTrCl or*tert*-butyldimethylsilyl chloride(TBSCl) in pyridine to furnish compounds **58** and **59** respectively.



Scheme 14: Synthesis of monosaccharide synthons **58** and **59**.

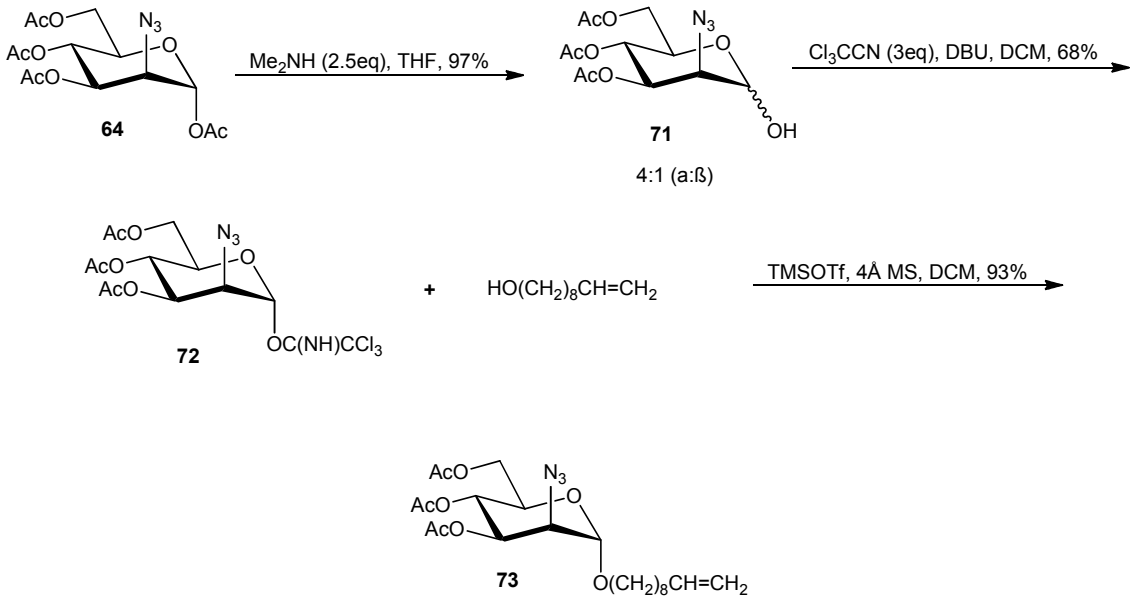
2.3.3 Synthesis of a monohydoxylic 9-decen-1-yl glycoside derivative

In order to produce the primary saccharide with linker attached a glycosylation was required. Initially it was thought that the thioglycosides could be used for the glycosyl donor in the glycosylation. A test reaction was carried out using synthon **57** as the donor and 9-decen-1-ol as the glycosyl acceptor (see **Scheme 15**).

Scheme 15: Attempted synthesis of decenyl glycoside using thioglycoside donor.

The N-iodosuccinimide (NIS) promoted glycosylation was carried out in the usual manor with a catalytic amount of silver triflate (AgOTf) and the addition of freshly activated molecular sieves.[78]The progress of the reaction was monitored with TLC, which showed consumption of the reactants but unfortunately an array of products were formed. It was thought that one possible reasonfor this issue could have been the iodonium ion generated from the NIS causing migration of the double bond of 9-decen-1-ol, however no products were isolated and no further analysis was performed.

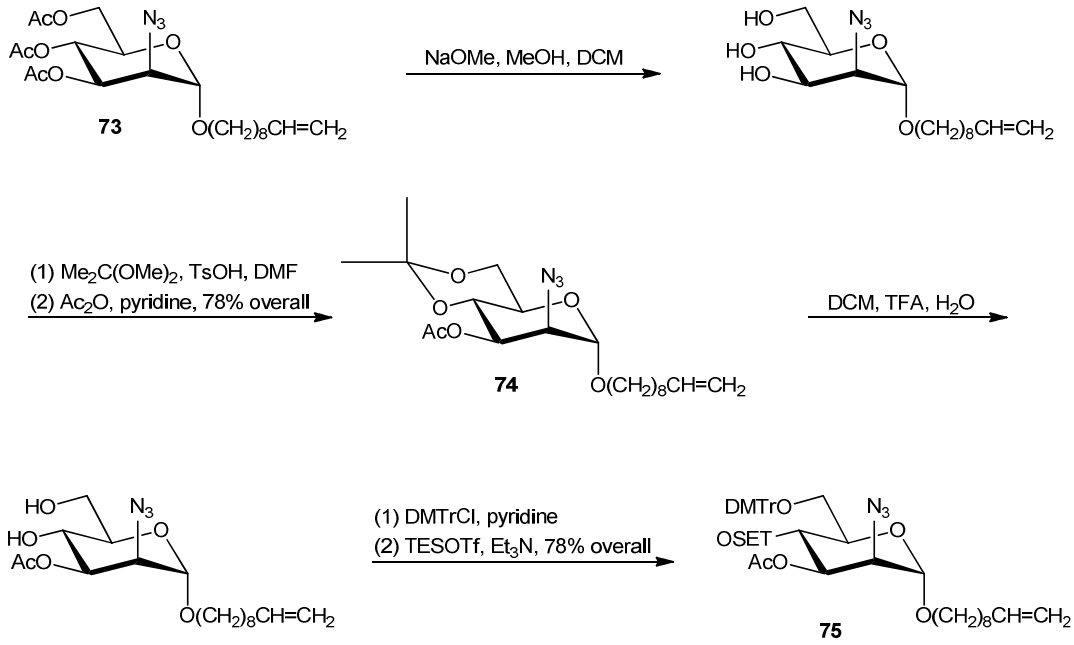
Instead a new approach was employed using the now plentiful tetra-acetate **64**. Firstanomeric deprotection of **64** was carried out by treatment with dimethylamine, which reacted quickly and efficiently to generate the hemiacetal **71** (see **Scheme 16**).



Scheme 16: Synthesis of 9-decen-1-yl glycoside using trichloroacetimidate donor.

After purification the hemiacetal could then be converted to the trichloroacetimidate glycosyl donor **72**through treatment with trichloroacetonitrile and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU).[110] The trichloroacetimidate donor was then used in a glycosylation[111,112] with 9-decen-1-ol as the acceptor and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf). This proceeded very rapidly with complete consumption of the donor after only ten minutes leaving the 9-decen-1-yl glycoside **73** in 93% yield.

With the glycosylation successful the next step was to install the correct protecting group arrangement. This was done following the same method as that used for the thioglycoside synthon **56** (**Scheme 17**).



Scheme 17: Synthesis of decenyl glycoside **75**.

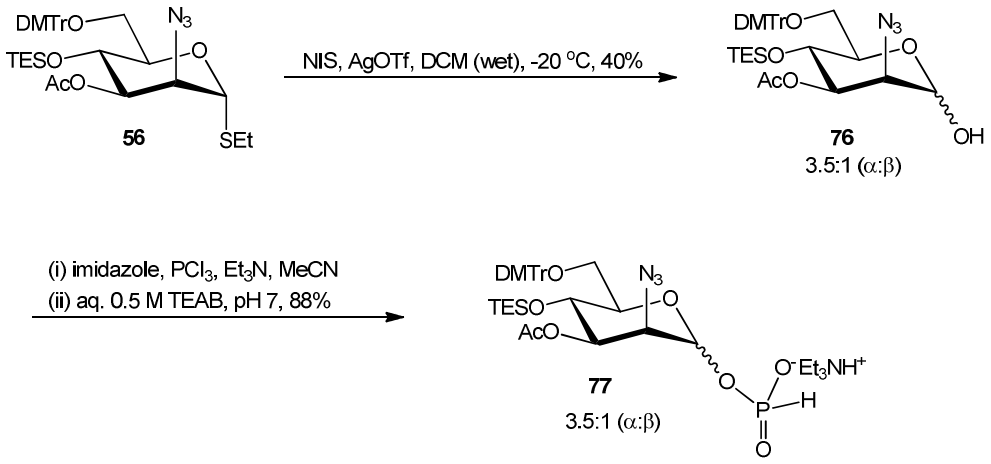
First the acetate groups were cleaved to release the triol before again using the isopropylidene ketal to temporarily protect the O-4 and O-6 positions. With this in place the required 3-O-acetyl group was installed to provide **74** in 78% yield. As before, after

acetylation, the ketal protection was cleaved with TFA releasing the 4,6-diol allowing the primary hydroxyl to be protected with a trityl ether followed by silylation of the O-4 position. After purification the required decenyl glycoside **75** was obtained with a 78% yield.

2.4 SYNTHESIS OF A GLYCOSYL H-PHOSPHONATE DERIVATIVE AND CONDENSATION REACTIONS

2.4.1 Synthesis of the H-phosphonate

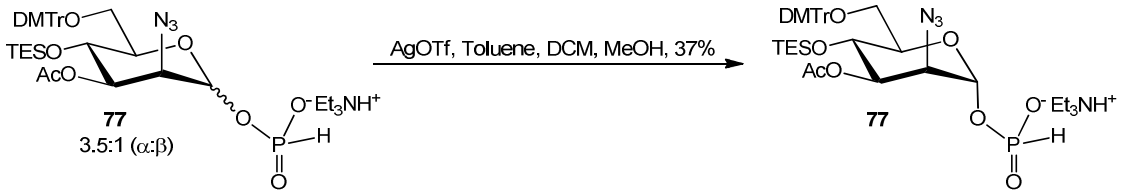
The initial work in this preparation was carried out using the prepared monosaccharide **56**. Hydrolysis of the ethyl thioglycoside **56** using NIS and AgOTf in a solution of wet dichloromethane[78] gave the desired hemiacetal **76** in a disappointing 40% yield and an α:β ratio of 3.5:1 (**Scheme 18**).



Scheme 18: Synthesis of DMTr protected H-phosphonate **77**.

Subsequent phosphonylation with phosphorus trichloride (PCl_3) and imidazole followed by aqueous work-up generated the H-phosphonate **77** efficiently in 88% yield. The H-

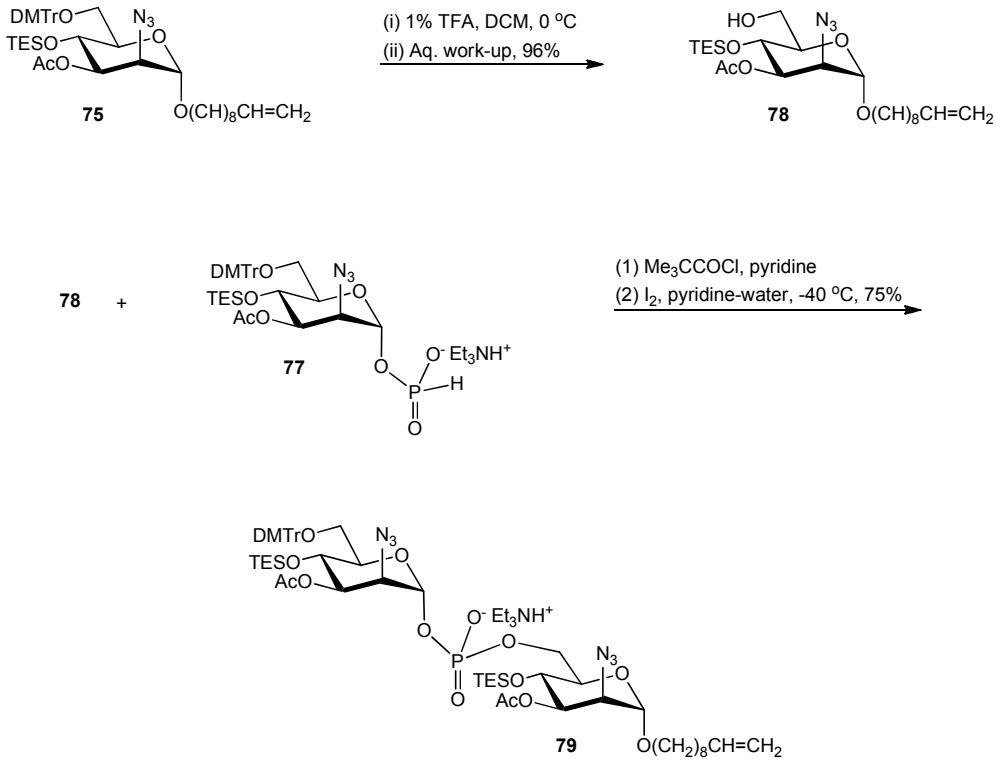
phosphonate formed however had, as expected, the same α:β ratio (3.5:1) as that of the previous hemiacetal. Before any coupling could proceed, this ratio had to be improved. This was achieved by the degradation of primarily the β-anomer with silver triflate (AgOTf) in a solution of toluene and methanol (**Scheme 19**). After purification the pure α-H-phosphonate **77** was isolated in 37% yield.



Scheme 19: Degradation of the β-H-phosphonate.

2.4.2 Condensation of H-phosphonate. The chain elongation up to a trisaccharide diphosphate derivative.

Following the successful synthesis of the H-phosphonate the first condensation was attempted. Prior to the coupling though the monohydroxylic acceptor **78** had to be generated (**Scheme 20)**. This was achieved by mild acid hydrolysis of the trityl ether from **75** using 1% (v/v) TFA in dichloromethane at 0 ^{o}C followed by aqueous work-up. This generated **78** (96%) with the required free hydroxyl at C-6 position.

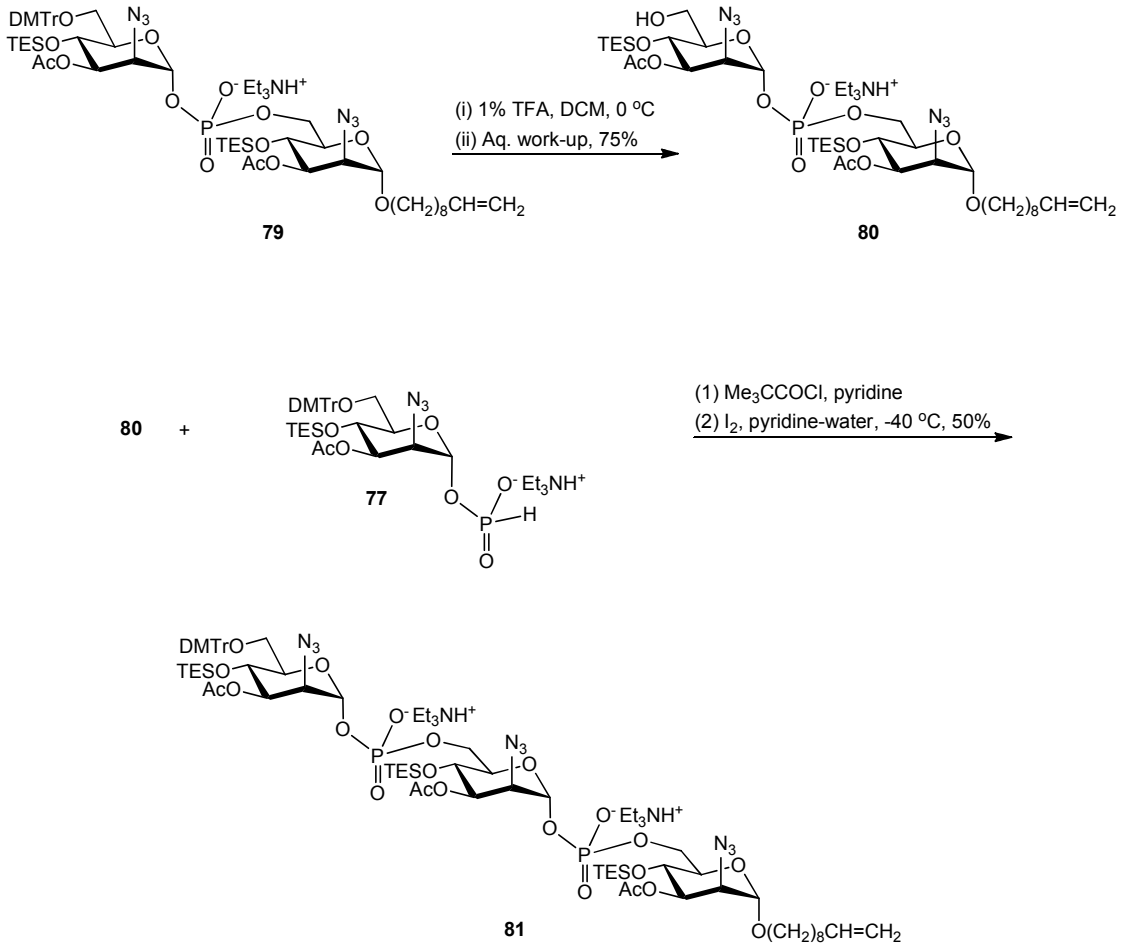


Scheme 20: Synthesis of disaccharide phosphate **79**.

The H-phosphonate **77** and the newly formed monohydroxylic derivative **78** were combined in pyridine and using standard H-phosphonate chemistry conditions the condensation was initiated with the addition of pivaloyl chloride.[80,88] Following complete consumption of the acceptor **78**, monitored by TLC, the reaction mixture was cooled to -40 ^{o}C before the formed H-phosphonic diester was oxidised with the addition of iodine in a solution of pyridine/water. The protected disaccharide phosphate **79** was then isolated in 75% yield.

To facilitate further chain elongation the trityl ether of the newly formed disaccharide phosphate **79** was cleaved using an ice-cold 1% (v/v) TFA-dichloromethane solution (**Scheme 21**).This led to the monohydroxylic derivative **80** in a 96% yield with no signs of degradation to the fragile phosphodiester linkage. After the successful deprotection the H-phosphonate **77** and the newly formed monohydroxylic disaccharide phosphate derivative **80** were combined in pyridine. The condensing

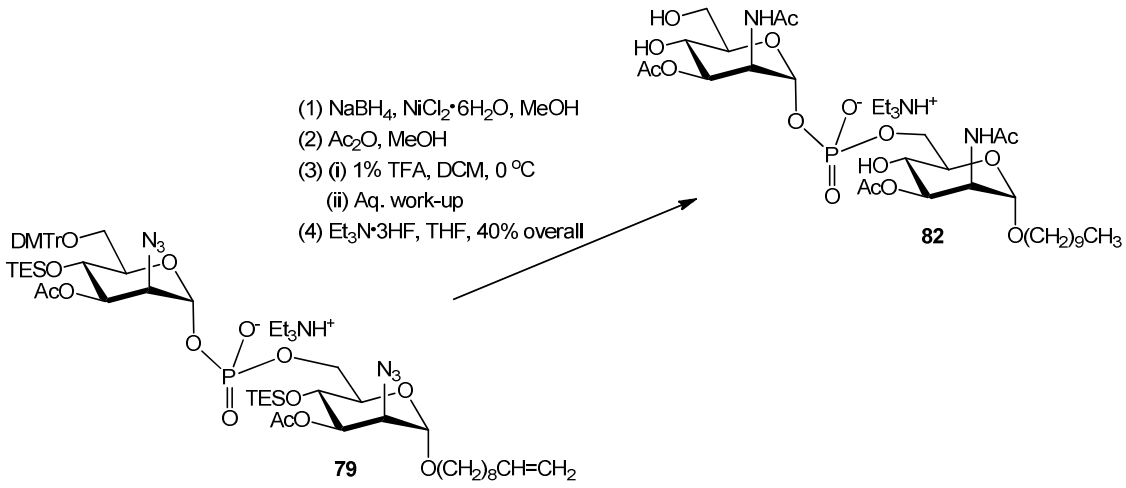
reagent pivaloyl chloride was then added to initiate the reaction. Following complete consumption of the acceptor **80**, again monitored by TLC, the mixture was cooled to -40 ^{o}C prior the addition of iodine in a solution of pyridine/water to facilitate further oxidation of the newly formed H-phosphonic diester. The trisaccharide diphosphate **81** was then isolated in 50% yield, showing a notable drop in efficiency for the introduction of the second intersaccharidic phosphate.



Scheme 21: Introduction of the second intersaccharidic phosphate.

With the formation of the disaccharide phosphate **79** and trisaccharide diphosphate **81** a success it deemed prudent to assess the suitability of the planned reduction and deprotection strategy. This involved initially the reduction of the azido functionality to

the required amine followed by *in situ* N-acetylation (**Scheme 22)**. This was carried out on the disaccharide phosphate **79** using sodium borohydride and nickel chloride hexahydrate as a catalyst in a solution of THF and methanol.



Scheme 22: Trial deprotection strategy.

Following the complete reduction of the azido groups, monitored by TLC, acetic anhydride was injected to accomplish N-acetylation. The trityl ether was then cleaved with a solution of TFA, as described earlier, before the removal of the triethylsilyl ethers using the $Et_3N{\cdot}3HF$ reagent in THF. This unfortunately led to the *n*-decanol derivative **82** (40%) after the unexpected reduction of the double bond, thus rendering the chosen 9-decen-yl linker ineffective.

To avoid this unwanted double bond reduction another method of reduction would have to be used. However, at this point only a small amount of material had been synthesised which had all been used in the initial deprotection attempt.It was therefore thought that this would be a good time to assess the synthetic strategy used to optimise the synthesis using the experience gained so far. As the double bond in the decenyl linker had been problematic in the first glycosylation attempt and also in the final deprotection, a

change in the nature of the linker was decided to avoid any further complications. It was also thought that the incorporation of the linker early in the synthesis complicated characterisation by adding a lot of additional signals into NMR spectra. This could easily be avoided for most of the synthesis by adding the linker onto the non-reducing end of the phosphoglycan in the final steps of the synthesis.

2.5 RE-DESIGNED SYNTHETIC STRATEGY

2.5.1 Revised synthetic targets

Due to the unforeseen issues encountered in the previous route a revised synthetic strategy was developed. Through this new strategy a revised set of synthetic targets was proposed.

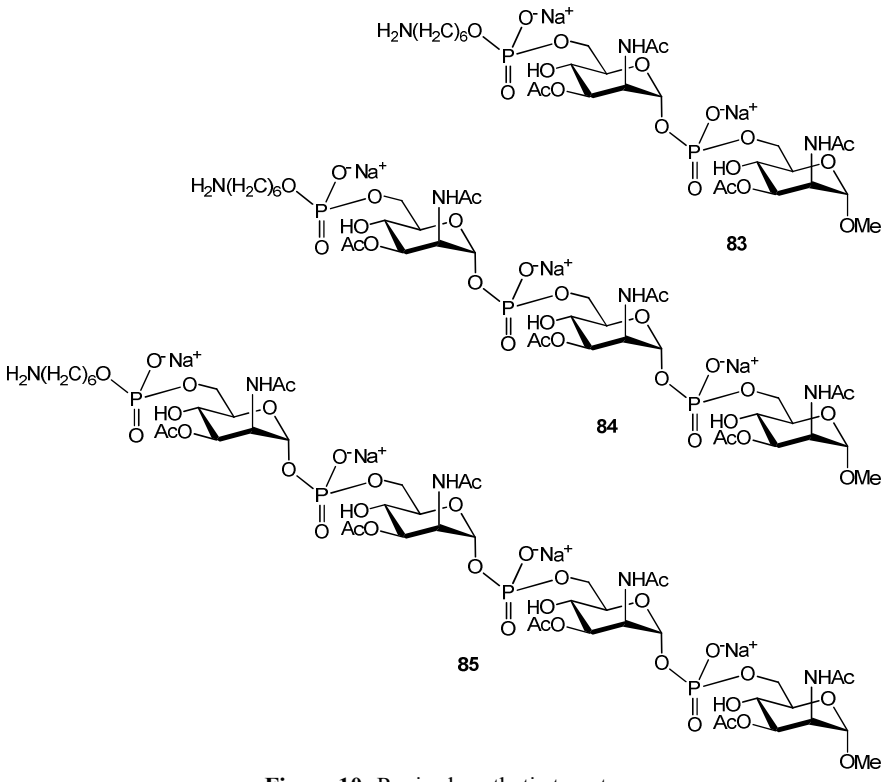
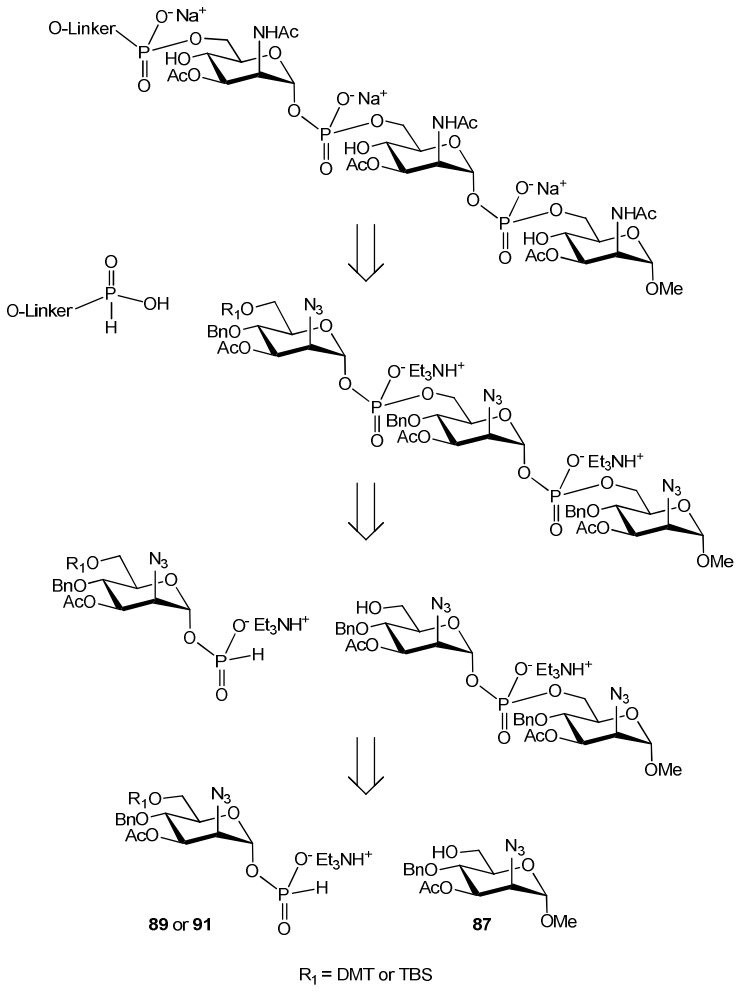


Figure 10: Revised synthetic targets.

As shown above, all of the synthetic targets **83-85** also contain an integrated 6-aminohexyl linker at the non-reducing end of the phosphoglycan chain allowing for conjugation of the synthetic CPS fragments to protein.

2.5.2 Revised retrosynthetic analysis

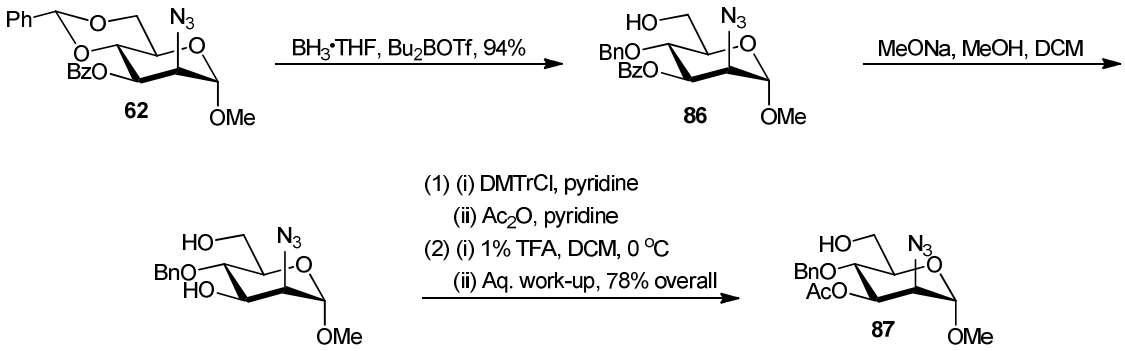
The new synthetic strategy focused on the use of synthons **58** and **59** and in place of the primary linker unit a simpler methyl glycoside **87** was proposed (**Scheme 23)**. This would allow the building of the fragments to the required size before finally capping the structure with a suitable 6-aminohexyl linker via a phosphate group at the non-reducing end of the phosphoglycan chain.



Scheme 23: Re-designed synthetic strategy.

2.5.3Synthesis of the methyl glycoside synthon 87

The first task for this revised strategy was the formation of the primary methyl mannoside unit **87** (**Scheme 24)**. This was accomplishedby the reductive cleavage[109] of the benzylidene derivative **62**, selectively opening the acetal ring at the O-6 position to give the monohydroxylic derivative **86**.



Scheme 24: Synthesis of primary saccharide synthon.

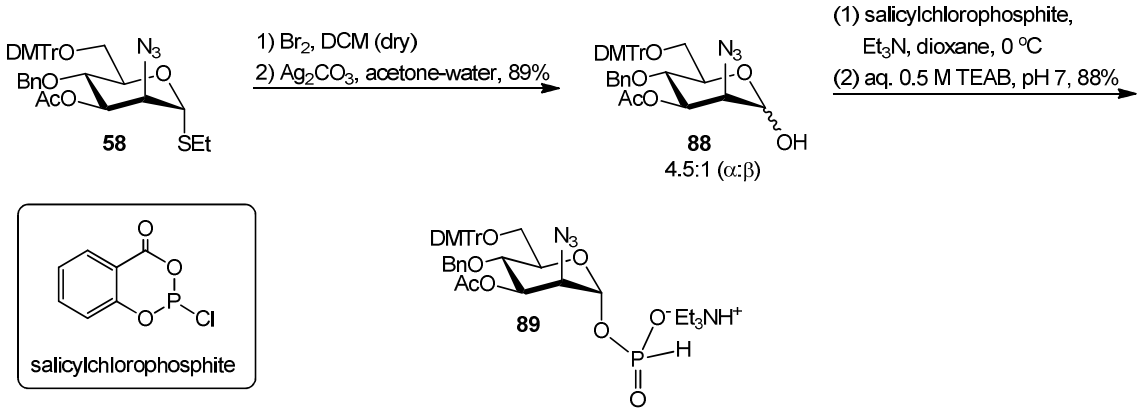
This was then treated with sodium methoxide and methanol to remove the benzoyl protection at the O-3position. The diol was then selectively tritylated at the O-6 position again using DMTrCl in pyridine after which the remaining O-3 hydroxyl was acetylated with the addition of acetic anhydride. The DMTr ether was then removed by treatment with an ice-cold solution of TFA in dichloromethane to furnish the desired methyl mannoside **87** in an overall yield of 78% over three steps.

2.6 IMPROVED SYNTHESIS OF THE GLYCOSYL H-PHOSPHONATES AND CONDENSATION REACTIONS

2.6.1 Synthesis of the H-phosphonates 89 and 91

During the assessment of the synthetic strategy it was noted that the hydrolysis of the thioglycoside **56**to form the hemiacetal**76** using NIS and AgOTf (**Scheme 18**)

was low yielding and unreliable. For this reason, in the new strategy, the hydrolysis was carried out by first reactingthe thioglycoside derivative **58** with bromine in dry dichloromethane to form a glycosyl bromide which was then hydrolysed to the hemiacetal**88***in situ* with the addition of Ag_2CO_3 and acetone-water (10:1)(**Scheme 25)**. This greatly improved the yield (to 89% from **58** to **88**) and proved a much more reliable route.

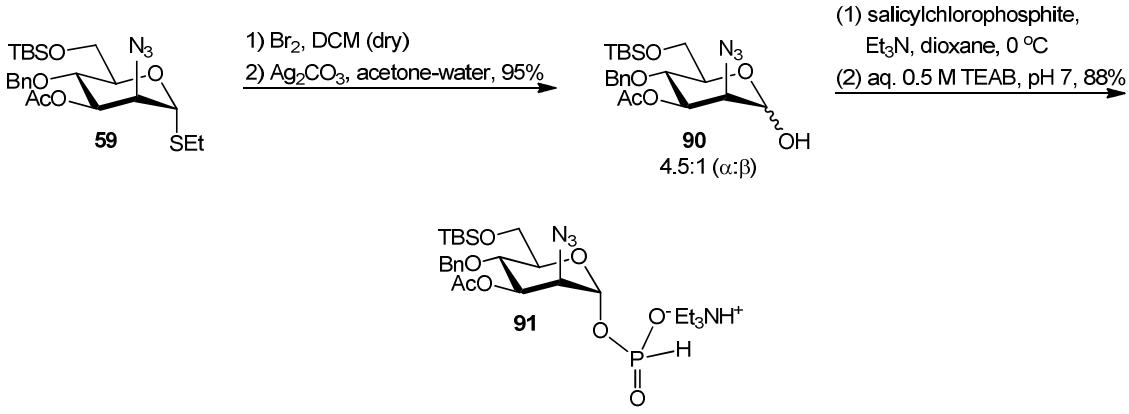


Scheme 25: Synthesis of the DMTr protected H-phosphonate **89**.

It was also thought at this point that the phosphonylation of the hemiacetal to the H-phosphonate using PCl_3 and imidazole was somewhat inefficient. Although the conversion to the H-phosphonate itself is a high yielding and effective reaction the stereo chemistry of the hemiacetal remains the same. In this case the α:βratio is 4.5:1 which leaves a high proportion of β-anomer which must be degraded, as seen in the previous synthetic route (**Scheme 19**). In order to avoid this issue a different phosphonylating reagent was utilised. By using salicylchlorophosphite[80,113]in a solution of dioxane-Et_3N,followed by aqueous work-up,the conversion of the hemiacetal **88**to the 6-O-DMTr protected H-phosphonate **89**proceeded with a very respectable yield of

88%, but most notably resulted an α:βratio of which only traces of the β-anomer were observed. This was an acceptable ratio to use without any further treatment.

The preparation of the TBS containing H-phosphonate derivative **91** also proceeded smoothly through the same route(**Scheme 26**). The thioglycoside **59** was treated with bromine in a solution of dichloromethane to facilitate the formation of a glycosyl bromide. Subsequentaddition of Ag_2CO_3 and acetone/water (10:1) enabled the *in situ*hydrolysis providing the hemiacetal**90** in an excellent 95% yield.



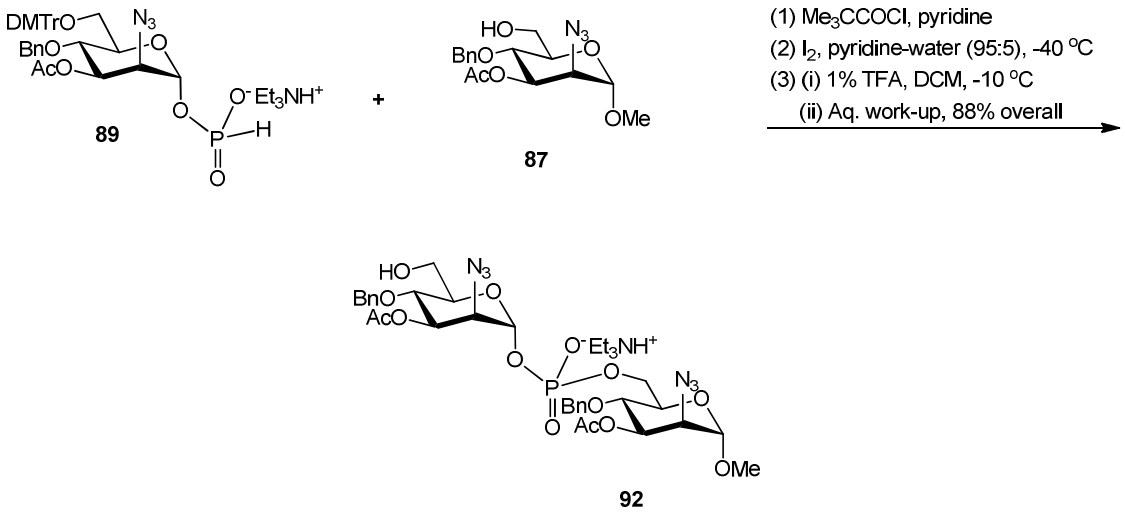
Scheme 26: Synthesis of the TBS protected H-phosphonate **91**.

Treatment of the hemiacetal **90** with salicylchlorophosphite in a solution of dioxane-Et_3N enabled the conversion to the6-O-TBSprotected H-phosphonate **91** with an 88% yield.

2.6.2 Condensation of the H-phosphonates. Synthesis of disaccharide phosphate derivatives.

The newly prepared primary methyl mannoside unit **87** was then combined with 6-O-DMTr protected H-phosphonate **89** in a solution of pyridine (**Scheme 27**). The condensation was initiated as before with the addition of pivaloyl chloride to the

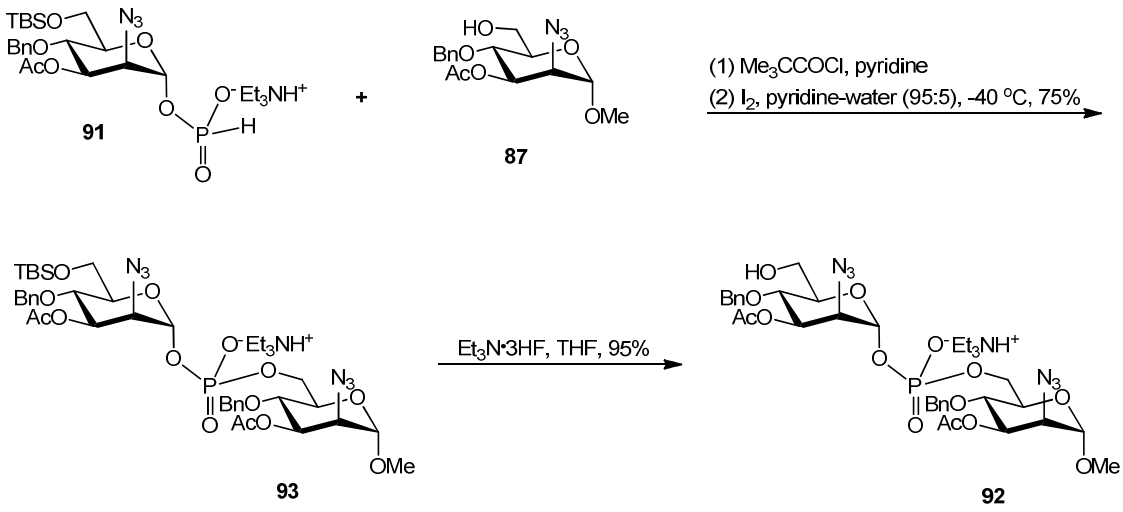
reaction mixture. Again the coupling was monitored by TLC to ensure the reactants were consumed and the condensation was complete.



Scheme 27: First condensation using DMTr protected H-phosphonate **89**.

In order to minimise potential cleavage of the fragile H-phosphonic diester bond during further oxidation[83,114] the reaction was cooled to -40 ^{o}C prior to a solution of pyridine/water and Et_3N being added. The oxidation was then initiated with the addition of solid iodine, which takes some 30 minutes to dissolve, giving slightly milder reaction conditions. The trityl ether was then removed using mild acid hydrolysis as described before again with a 1% (v/v) TFA/dichloromethane solution but to minimise risk to the newly formed phosphodiester the temperature was dropped to -10 ^{o}C. The disaccharide phosphate **92** was then isolated in 88% yield.

Following the successful high yielding synthesis of disaccharide phosphate **92** usingthe dimethoxytrityl protected H-phosphonate **89**the condensation was repeated this time with the silyl protected H-phosphonate **91 (Scheme 28)**.



Scheme 28: First condensation using TBS protected H-phosphonate **91**.

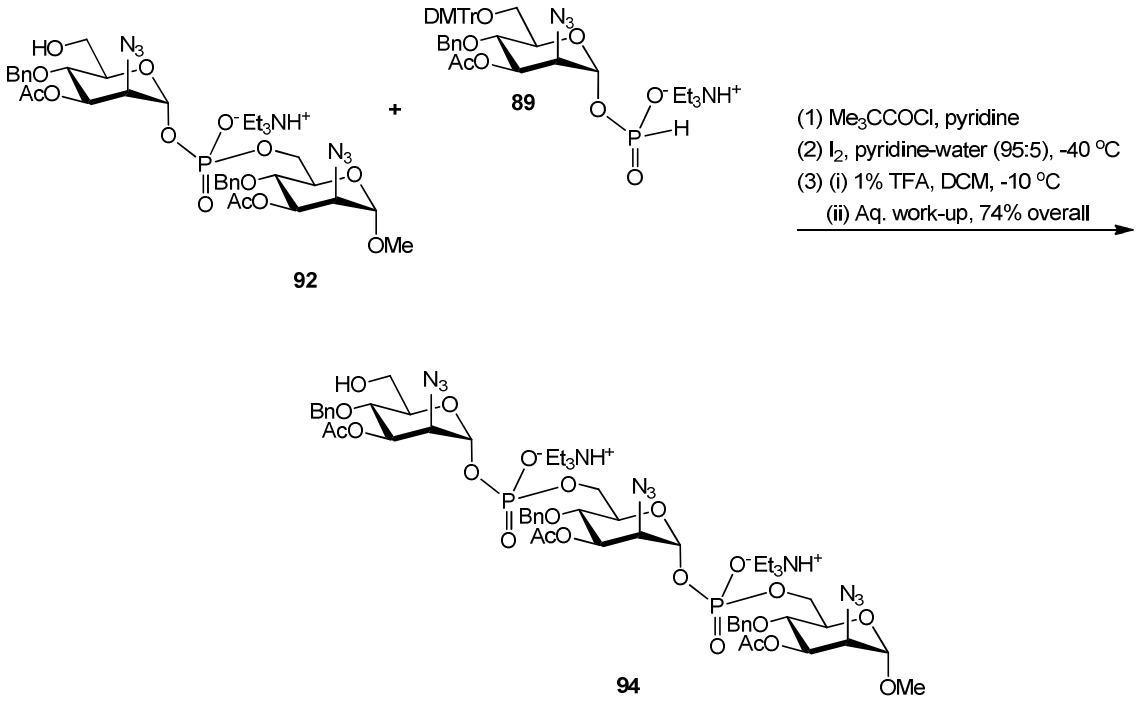
The primary methyl mannoside unit **87** was combined this time with the TBS protected H-phosphonate **91** in a solution of pyridine. Pivaloyl chloride was added to the reaction mixture as before to initiate the condensation. Coupling was monitored by TLC until all of derivative **87** was consumed.Again to prevent any potential cleavage of the fragile H-phosphonic diester bond during the oxidation, the reaction was cooled to -40 ^{o}C before a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine which again took some 30 minutes to fully dissolve at the reduced temperature. The disaccharide phosphate **93** was then isolated in 75% yield but still contained the TBS protecting group at the O-6' position. In order for the TBS protection to be cleaved derivative **93** was first dissolved in a solution of tetrahydrofuran (THF) before addition of triethylamine tri(hydrogen fluoride) (Et_3N•3HF). This facilitated cleavage of the silyl protection to reproduce the previously synthesised disaccharide phosphate **92** in a 95% yield.

Although successful, the synthesis of the disaccharide phosphate **92** through the use of the TBS protected H-phosphonate **91** was slightly less efficient. The condensation itself was somewhat lower yielding, 75% using the TBS H-phosphonate

91compared with the 88% (after consecutive condensation, oxidation and detritylation steps) for the DMTr protected H-phosphonate **89**. The O-6' desilylation step also required a lot longer reaction time and used more hazardous reagents compared with the detritylation step. For these reasons it was decided that the DMTr protected H-phosphonate **89** was the best candidate to proceed with all further chain elongations.

2.6.3 Synthesis of a trisaccharide diphosphate derivative.

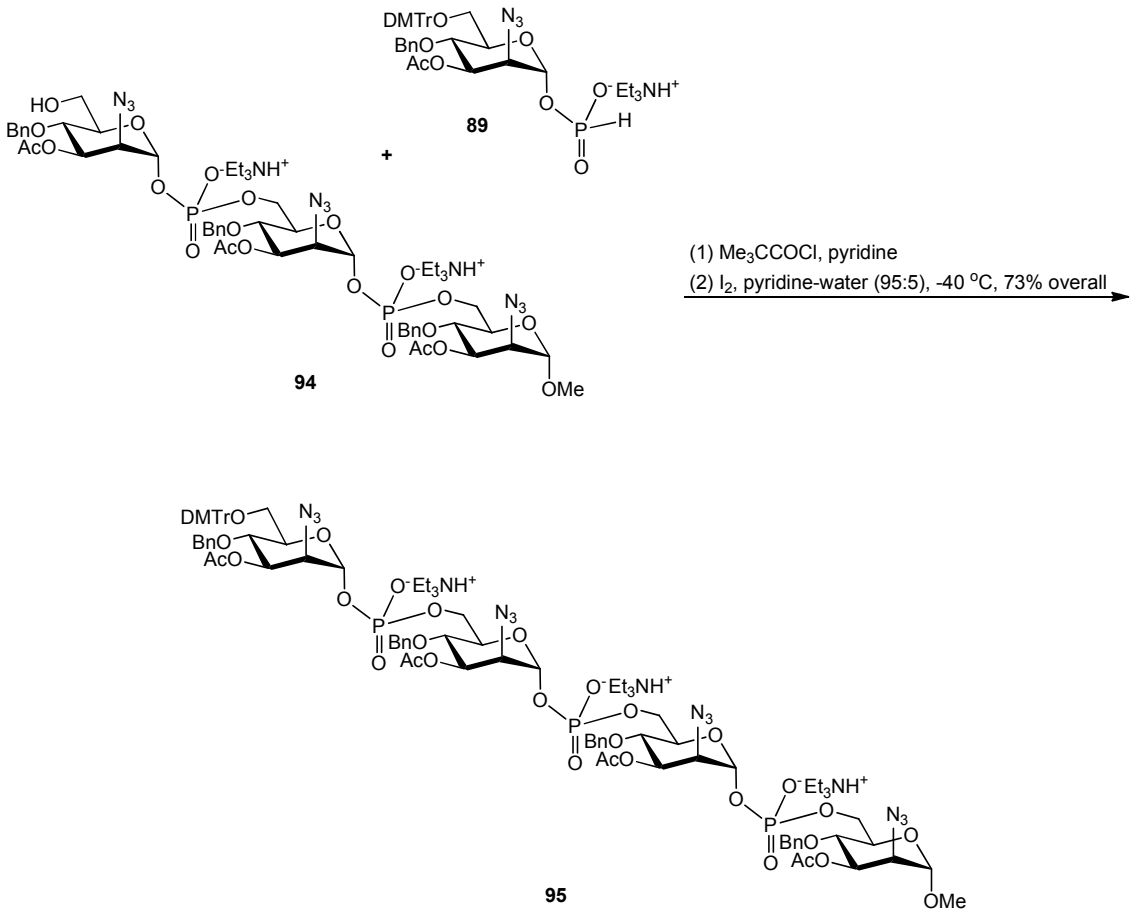
After the successful synthesis of the disaccharide phosphate **92**,which already contained the required C-6' hydroxyl it was possible to proceed straight to the introduction of a second intersaccharidic phosphate. The monohydroxylic disaccharide phosphate **92** was combined with the DMTr protected H-phosphonate derivative **89** (**Scheme 29**)in pyridine and condensation was again initiated with pivaloyl chloride.The reaction was cooled to -40 ^{o}C prior to a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine. The trityl ether was then removed using mild acid hydrolysis with a 1% (v/v) TFA/dichloromethane solution at a reduced temperature of -10 ^{o}C. The trisaccharide diphosphate **94** was then isolatedin a very good yield of 74%. It should be mentioned that through this strategy only a slight decrease in yield was observed when the second intersaccharidic phosphate is introduced.



Scheme 29: Introduction of the second intersaccharidic phosphate.

2.6.3Synthesis of a tetrasaccharide triphosphate derivative.

Now with the trisaccharide diphosphate **94**in hand it was possible to attempt the introduction of the third intersaccharidic phosphate (**Scheme 30**). The trisaccharide diphosphate **94** andthe H-phosphonate **89** were combined in pyridine prior to the addition of pivaloyl chloride to begin the condensation. The oxidation was again performed at -40 ^{o}C, initiated with the addition of solid iodine, in a solution of pyridine/water. As this was the first attempt at the introduction of a third intersaccharidic phosphate, in order to avoid any potential phosphodiester cleavage the acidic conditions required for detritylation were avoided (at this stage of the project) and the DMTr protected tetrasaccharide triphosphate **95** was isolated in a very impressive yield of 73%. So, the first synthesis of a linear ManNAc phosphate oligomer containing three intersaccharidic phosphates (see **Figure 12**) was performed successfully.



Scheme 30: Introduction of the third intersaccharidic phosphate.

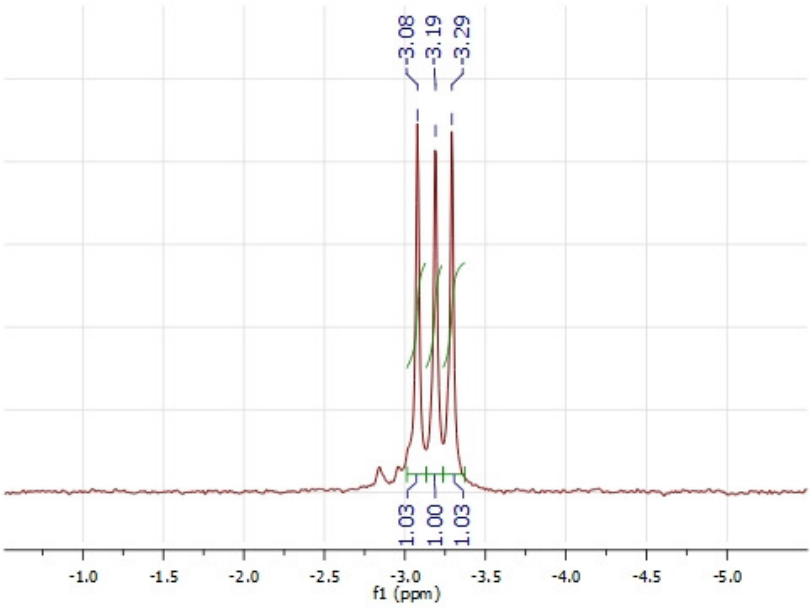
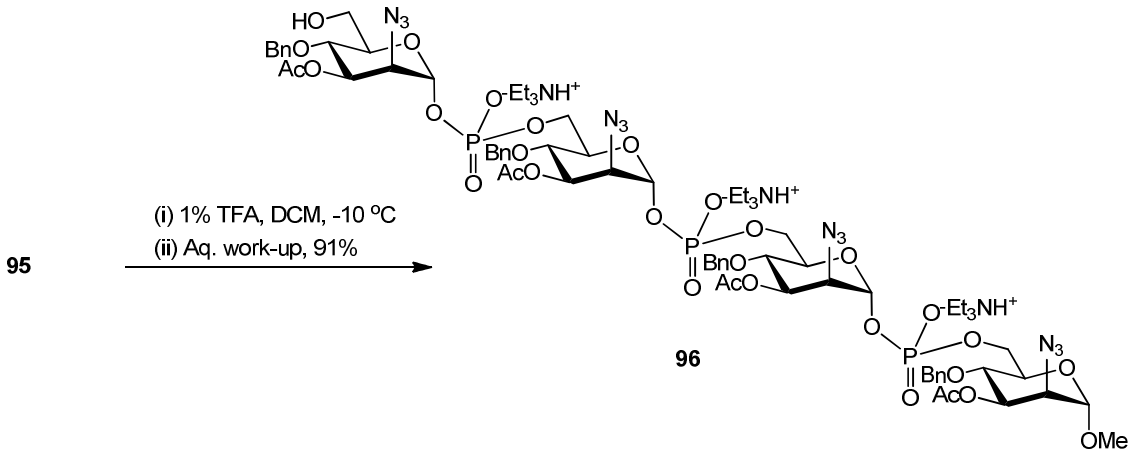


Figure 12: ^{31}P NMR of DMTr protected tetrasaccharide triphosphate **95.**

Now, having shown the condensation and oxidation had progressed without issue for the introduction of a third intersaccharidic phosphate, it was time to assess the

stability during detritylation now that the phosphoglycan contained 3 fragile phosphodiester linkages (**Scheme 31**).



Scheme 31: Detritylation of the tetrasaccharide triphosphate **95**

This was achieved by mild acid hydrolysis of the trityl ether from **95** using 1% (v/v) TFA in dichloromethane at -10 ^{o}C followed by aqueous work-up. This generated **96** (96%) with the required free hydroxyl at C-6''' position, causing a characteristic change to the ^{31}P chemical shift (see **Figure 13**), necessary for integration of the linker and possibly further chain elongation.

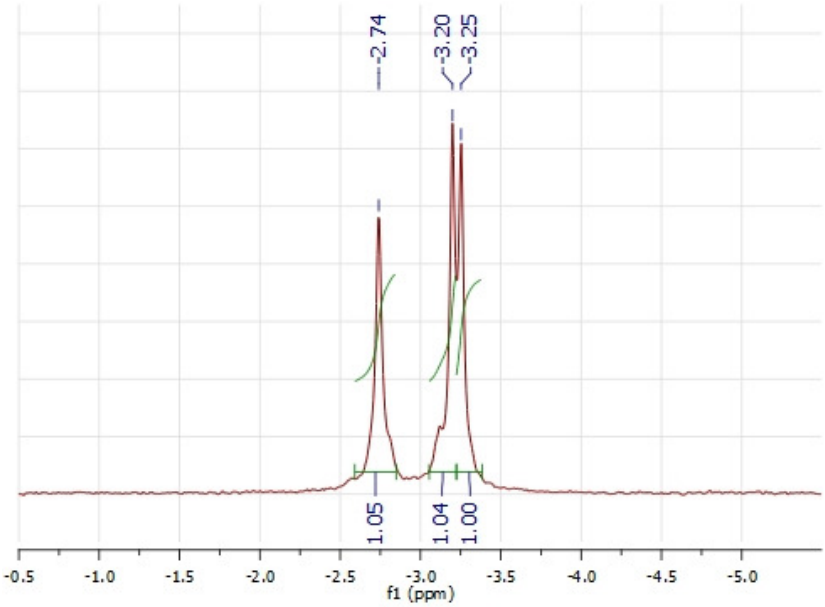
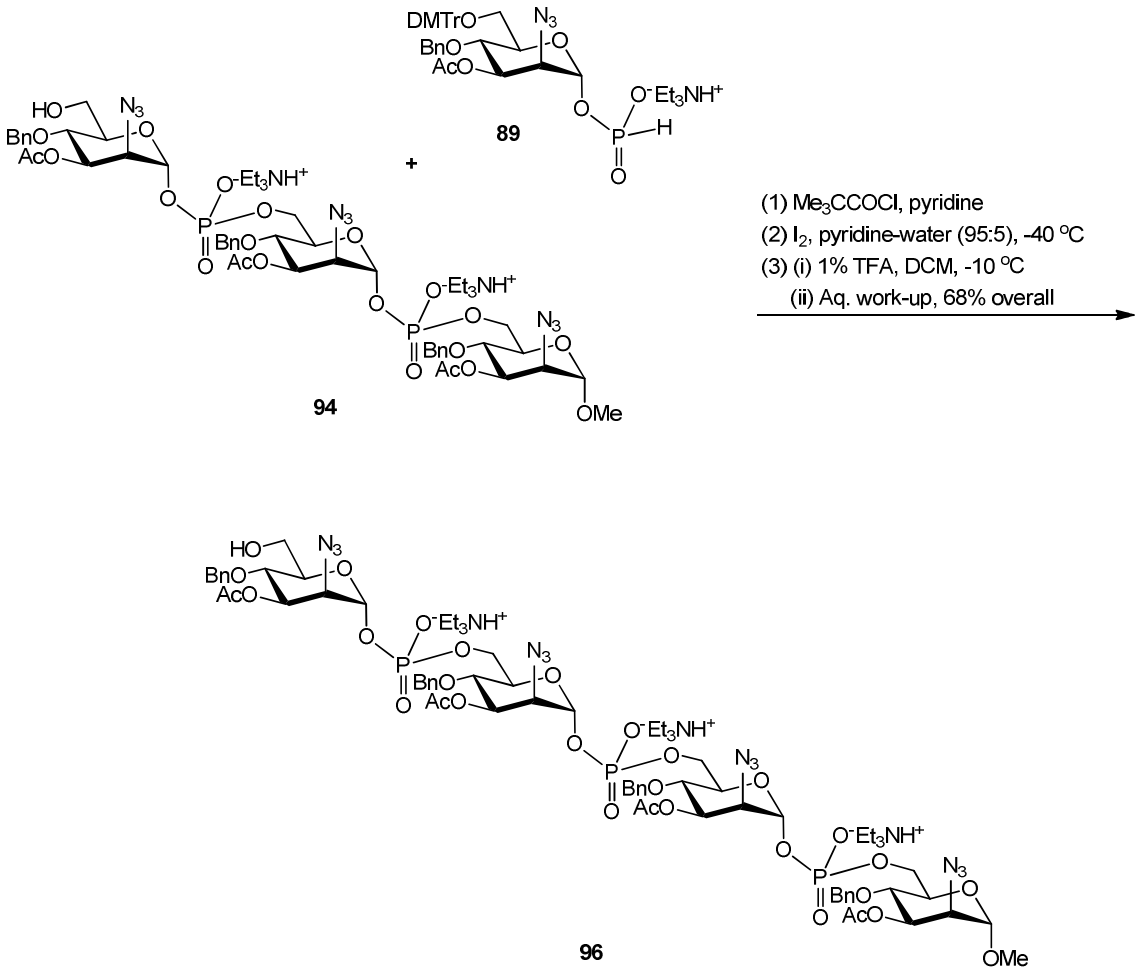


Figure 13: ^{31}P NMR of tetrasaccharide triphosphate **96.**

It was now known that the condensation followed by oxidationto form thetetrasaccharide triphosphate **95**worked well with no obvious signs of degradation. Further, detritylationunder mild acidic conditions was also shown to proceed with no signs of cleavage of the fragile phosphodiester linkage. With this in mind the preparation was repeated using the same 3 step process as that used for the preparation of the disaccharide phosphate **92** and the trisaccharide diphosphate **94** (**Scheme 32**).



Scheme 32: Introduction of a third intersaccharidic phosphate with 6’’’-O-deprotection.

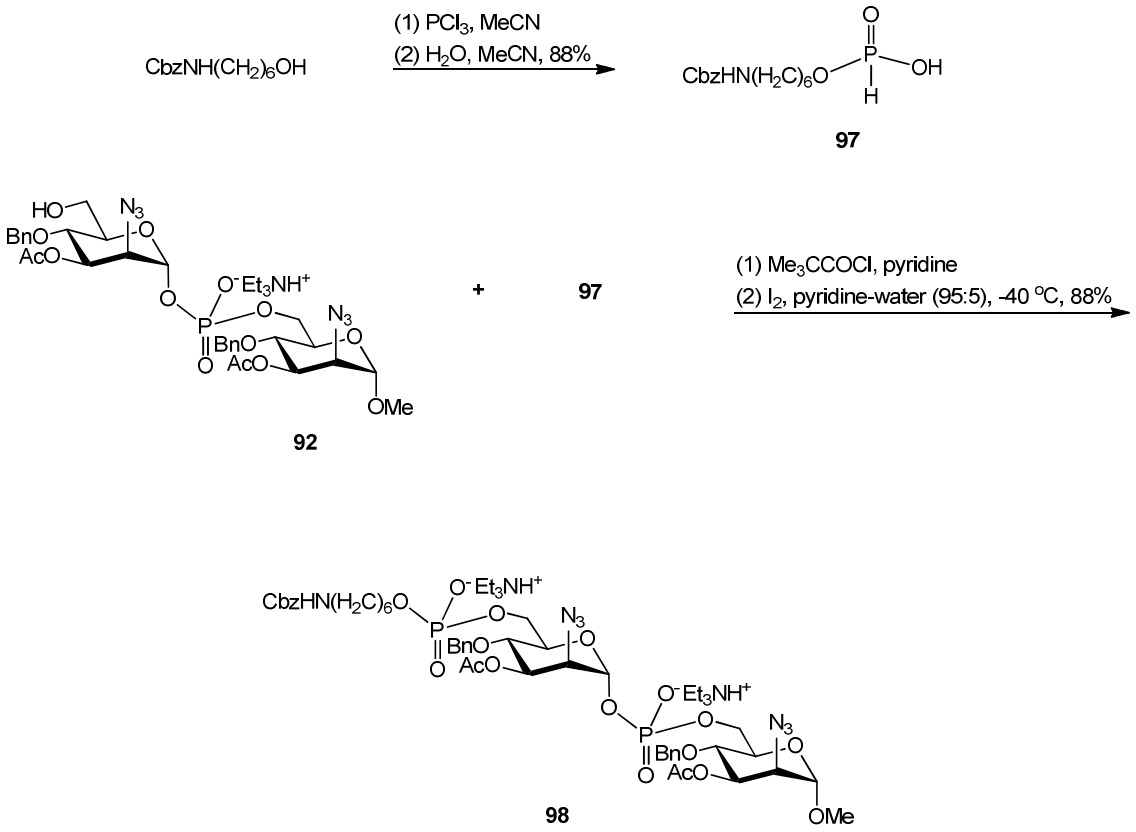
The monohydroxylic disaccharide phosphate **94** was combined with the DMTr protected H-phosphonate derivative **89** in pyridine and condensation was again initiated with pivaloyl chloride.The reaction was cooled to -40 ^{o}C before a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine. Detritylation was achieved by mild acid hydrolysis with a 1% (v/v)

TFA/dichloromethane solution at a reduced temperature of -10 ^{o}C. The tetrasaccharide triphosphate **96** was then isolatedin a very good yield of 68% overall, showing that the synthesis is both efficient and reproducible.

2.6.4 Synthesis of linker containing phosphosaccharide derivatives.

In order to allow for future conjugation of the synthesised phosphosaccharides to protein a suitable linker was required. The linker chosen in this case was 6-aminohexanol, the amine moiety of which was protected with a benzyloxycarbonyl (Cbz) group (**Scheme 33**). The introduction of the linker was to the non-reducing end of the molecule througha phosphodiester bridge, ruling out a simple glycosylation, as used earlier for the introduction of the dec-9-enyl linker. The linker had to be converted to a more reactive species first before introduction could be realised and an ideal candidate for this was a 6-aminohexyl H-phosphonate derivative. This strategy would add the linker to the phosphoglycans whilst also introducing an additional phosphate moiety.

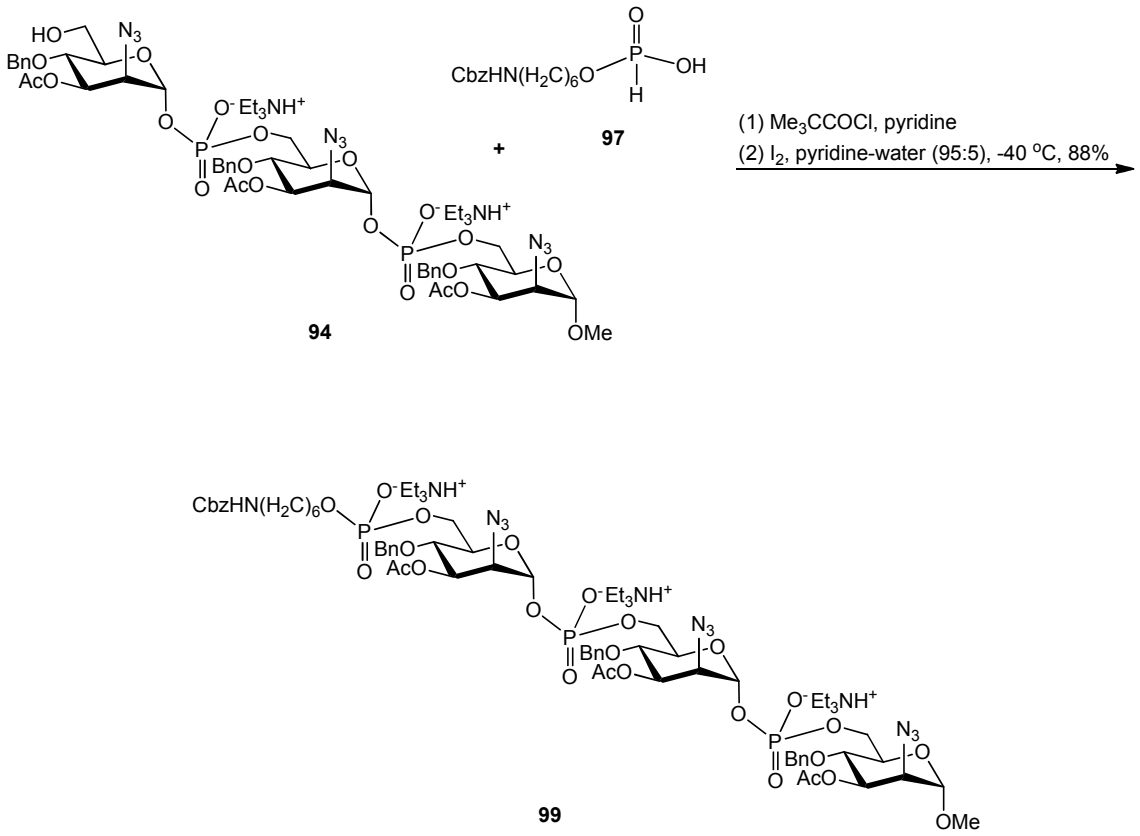
The commercially available Cbz protected 6-amino-hexanol was first converted to the H-phosphonate through treatment with phosphorous trichloride followed by aqueous work-up to yield the H-phosphonate linker derivative **97** in 88% yield.



Scheme 33: Synthesis and condensation of the linker H-phosphonate **97**.

The disaccharide phosphate **92** was combined with the H-phosphonate **97** in pyridine and condensation was again initiated with pivaloyl chloride.The reaction was then cooled to -40 ^{o}C after which time a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine.The linker containing disaccharide diphosphate **98** was isolated in a very impressive yield of 88%.

The H-phosphonate linker **97** was again used for the introduction of the linker to the trisaccharide diphosphate **94** (**Scheme 32**). This again provided the required linker whilst also introducing a third phosphate moiety to the CPS fragment.



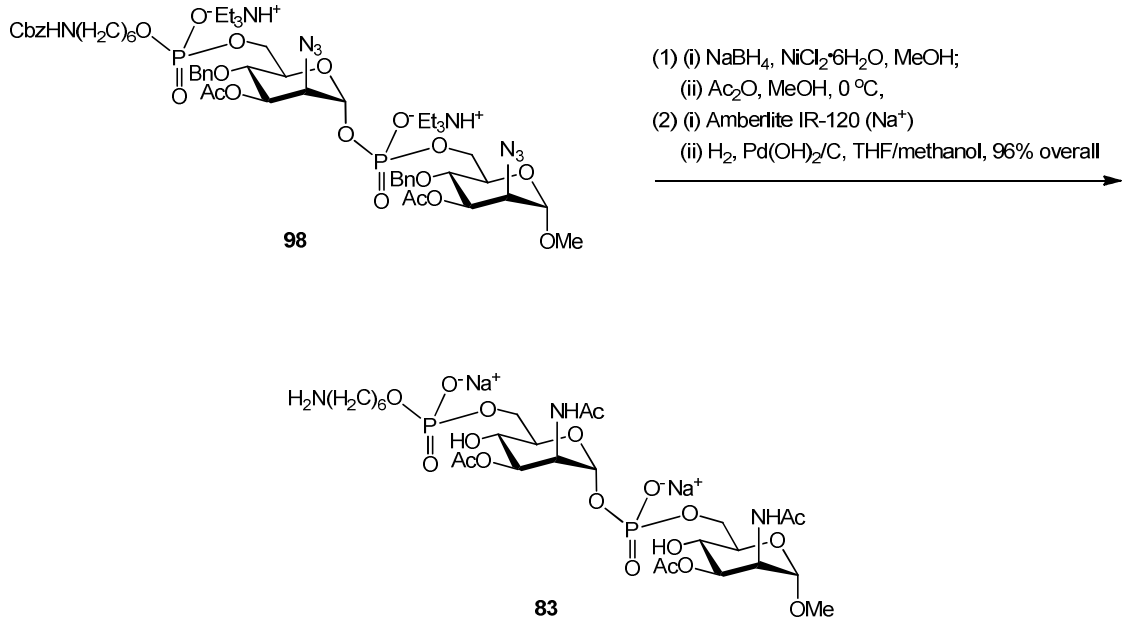
Scheme 34: Condensation of linker H-phosphonate.

The trisaccharide diphosphate **94** was combined with the H-phosphonate **97** in pyridine before addition of pivaloyl chloride to initiate the condensation.Following coupling, the reaction was cooled to -40 ^{o}C after which time a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine.The linker containing trisaccharide triphosphate **99** was isolated in a very impressive yield of 88%.

2.6.5 Deprotection of linker containing phosphosaccharide derivatives 98 and 99

With the successful formation of the disaccharide diphosphate **98** and trisaccharide triphosphate **99** it was time to assess the suitability of the planned reduction and deprotection strategy. This involved initially the reduction of the azido functionality to the required amino group followed by *in situ* N-acetylation (**Scheme35**). This was carried out on the disaccharide diphosphate **98** using sodium

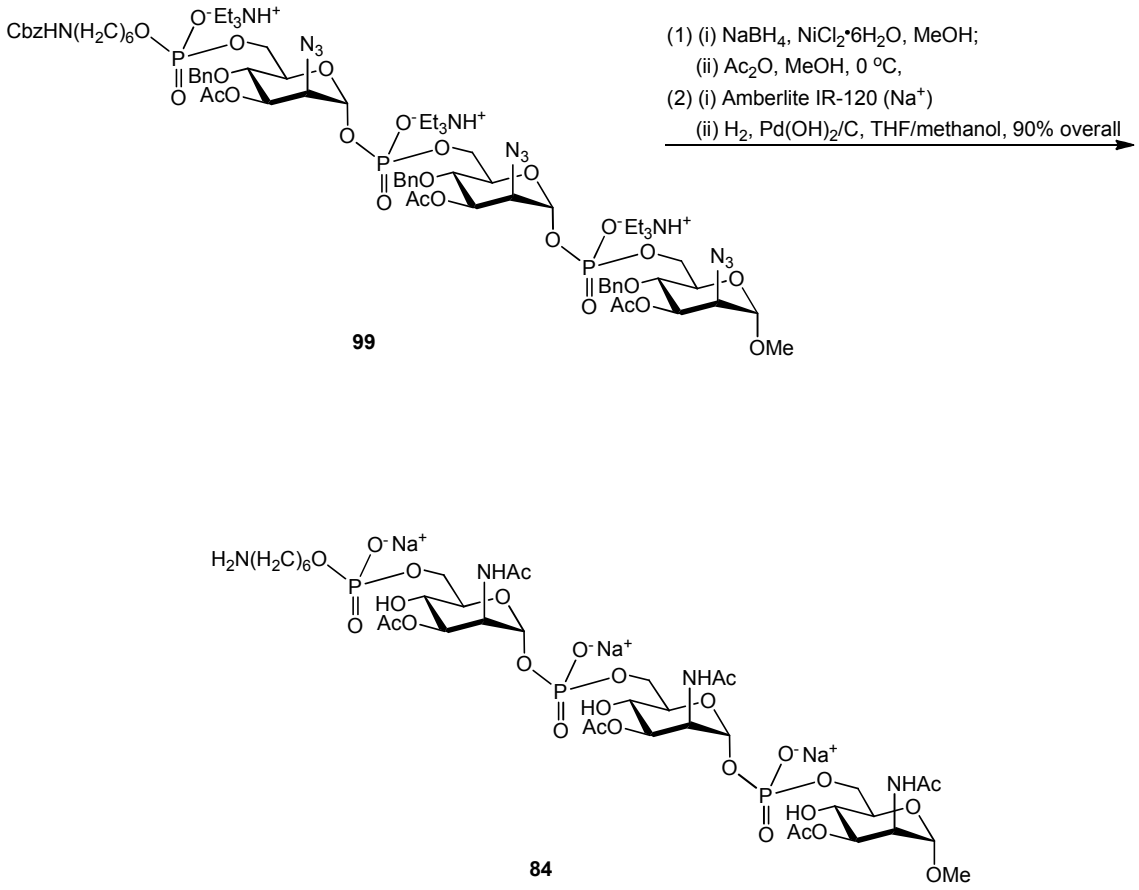
borohydride and nickel chloride hexahydrate as a catalyst in a solution of THF and methanol.



Scheme 35: Deprotection to provide synthetic target **83**.

Following the complete reduction of the azido groups, acetic anhydride was injected to undertake N-acetylation. The remaining O-4 benzyl protection and Cbz protection of the linker could then be removed through catalytic hydrogenation. This was achieved by first treating with IR-120 (Na^+) ion exchange resin to convert the phosphate triethylammonium salts to the corresponding di-sodium salt which is known to help facilitate the hydrogenation and increase the overall efficiency of the reaction.[81,115]The prepared disaccharide diphosphatewas then hydrogenated over palladium hydroxide on charcoal ($Pd(OH)_2/C$) in a solution of methanol/THF to provide the first synthetic target **83** in 96% yield overall.

Given that the deprotection of the disaccharide diphosphate **98** proceeded without issue, it was possible to continue straight to the deprotection of the trisaccharide triphosphate **99** with the same reaction conditions (**Scheme 36**).



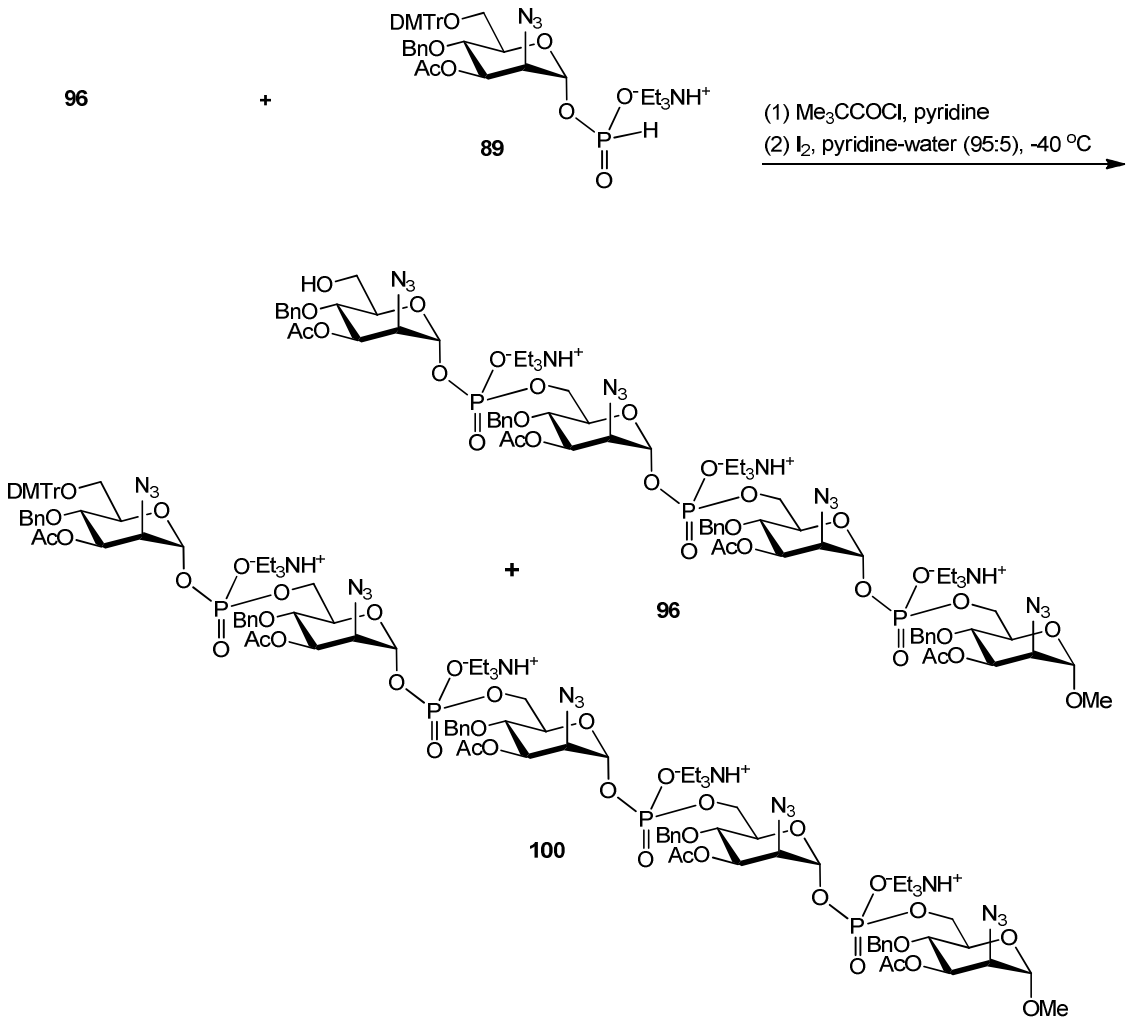
Scheme 36: Deprotection to provide synthetic target **84**.

The trisaccharide triphosphate **99** was dissolved in a solution of THF and methanol and subsequent reduction was performed with the addition of sodium borohydride and nickel chloride hexahydrate as a catalyst. Following reduction of the azido groups, acetic anhydride was again introduced to accomplish N-acetylation. Conversion of the phosphates to the sodium salt form was achieved by treating with IR-120 (Na^+) ion exchange resin. The remaining benzyl and Cbz protecting groups were then removed through catalytic hydrogenolysis over palladium hydroxide on charcoal ($Pd(OH)_2/C$) in

a solution of methanol/THF to provide the second synthetic target **84**in 90% yield overall.

2.6.6 Attempted chain elongation of a tetrasaccharide triphosphate derivative 96.

Encouraged by the high yielding success for the synthesis of the tetrasaccharide triphosphate **96**,a further condensation was attempted to introduce a fourth intersaccharidic phosphate (**Scheme 37**).



Scheme 37: Attempted introduction of a fourth intersaccharidic phosphate.

To this end, the tetrasaccharide triphosphate **96** (a monohydroxylic derivative)andthe H-phosphonate **89** were then combined in pyridine prior to the addition of pivaloyl

chloride to begin the condensation. Coupling was again monitored with TLC however analysis of progress was somewhat complicated. The monohydroxylic acceptor **96**, already containing three phosphate moieties, and the pentasaccharide tetraphosphate product **100** had identical travel (i.e., R_f values) on the TLC plate. The condensation itself was definitely occurring as seen by orange colour produced by the dimethoxytrityl group of thepentasaccharide tetraphosphateformed on developing the TLC plate. The main problem was estimating how much of the monohydroxylic acceptor **96** had been consumed. After 40 minutes of condensation conditions some other minor spots could be seen on the TLC plate, probably indicating signs of degradation. At this point it was judged best to proceed with the oxidation. The oxidation was again performed at -40 ^{o}C, initiated with the addition of solid iodine, in a solution of pyridine/water. This gave, after purification, an inseparable mixture (~1:1) of the newly formed pentasaccharide tetraphosphate **100** and tetrasaccharide triphosphate **96** starting material.

At this stage it was thought that the best course of action for this inseparable mixture was to attempt the condensation again to convert the remaining monohydroxylic acceptor **96** to thepentasaccharide tetraphosphate **100**. The inseparable mixtureandthe H-phosphonate **89** were then combined in pyridine and pivaloyl chloride was added to begin the condensation. The progress again was difficult to monitor but the reaction conditions were maintained until minor signs of degradation were observed. The oxidation was performed at -40 ^{o}C, initiated with the addition of solid iodine, in a solution of pyridine/water. Unfortunately this additional condensation did not appear to improve the situation as again an inseparable mixture (~1:1) of the pentasaccharide tetraphosphate **100** and tetrasaccharide triphosphate **96** starting material was isolated (see **Figure 14**).

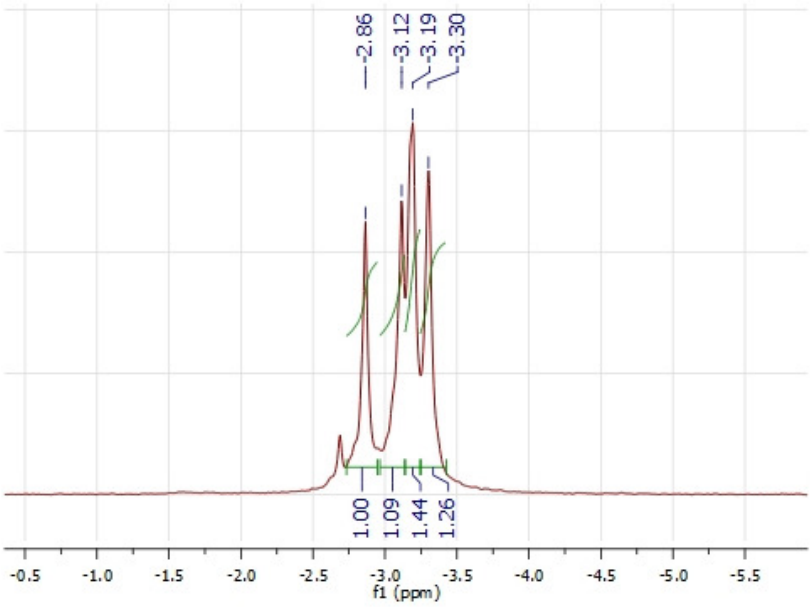


Figure 14: ^{31}P NMR of inseparable mixture of tetrasaccharide triphosphate **96** andpentasaccharide tetraphosphate **100.**

The mixture of tetraphosphate **100** and triphosphate **96** was then detritylated under mild acid hydrolysis conditions, 1% (v/v) TFA/dichloromethane solution at a reduced temperature of -10 ^{o}C (**Scheme 38**). The resulting mixture generated five distinct peaks, in a ratio of 2:2:1:1:1, with ^{31}P NMR analysis (see **Figure 15**).

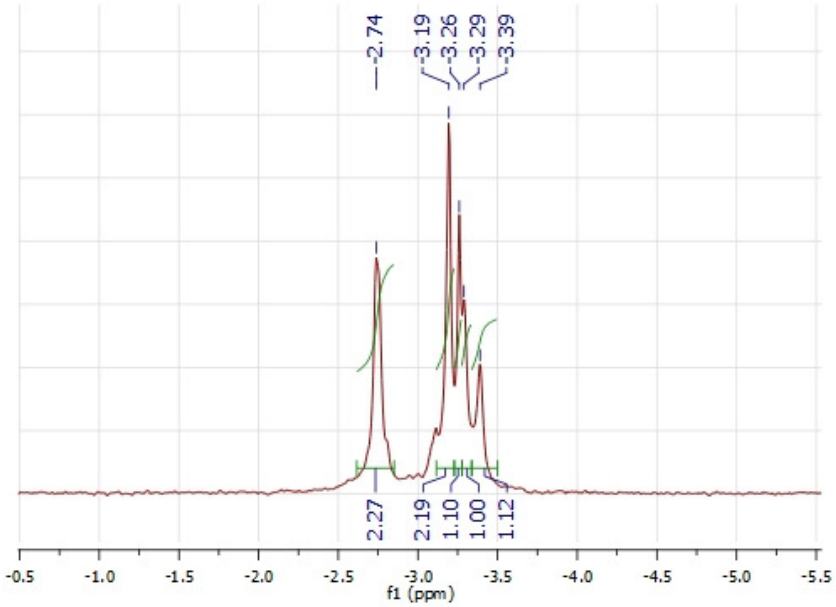
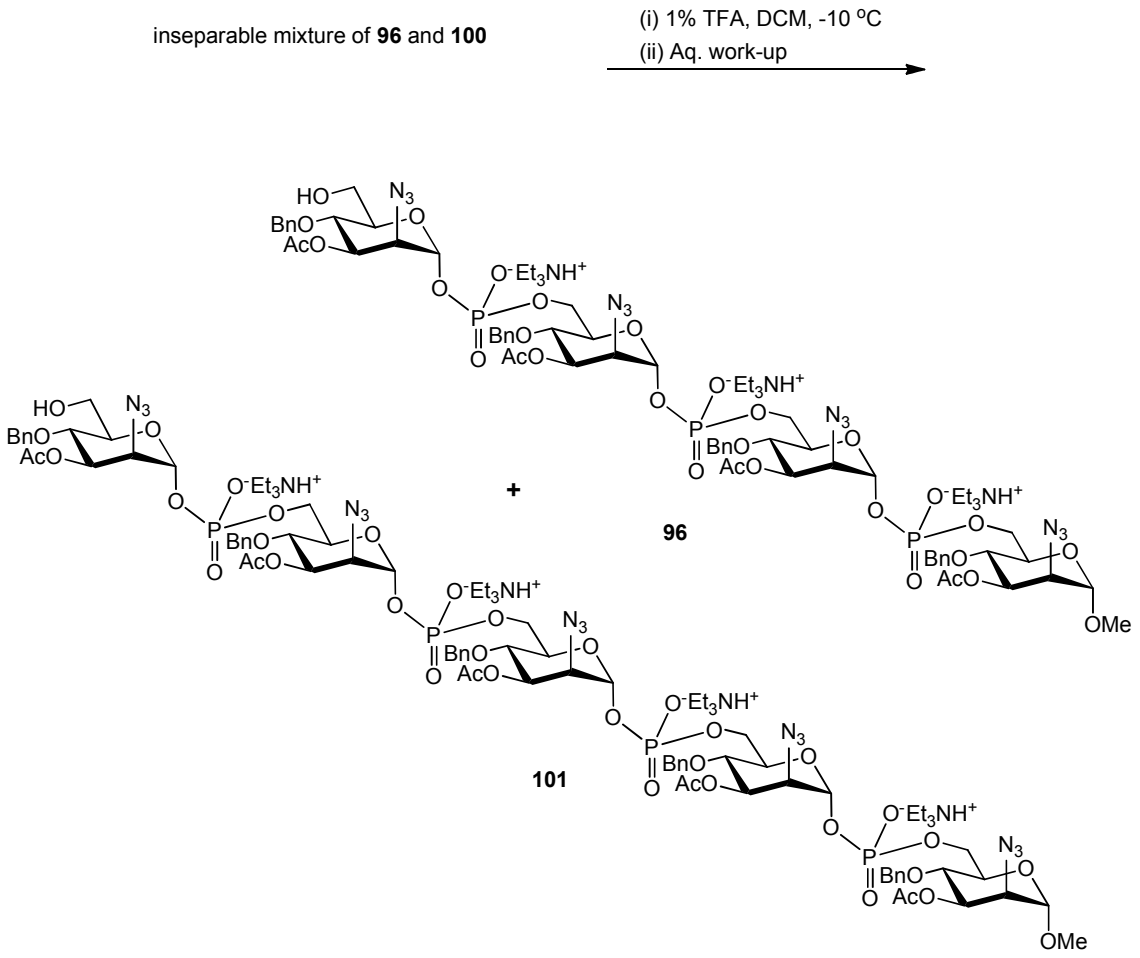


Figure 15: :^{31}P NMR of inseparable mixture of tetrasaccharide triphosphate **96** andpentasaccharide tetraphosphate **101.**

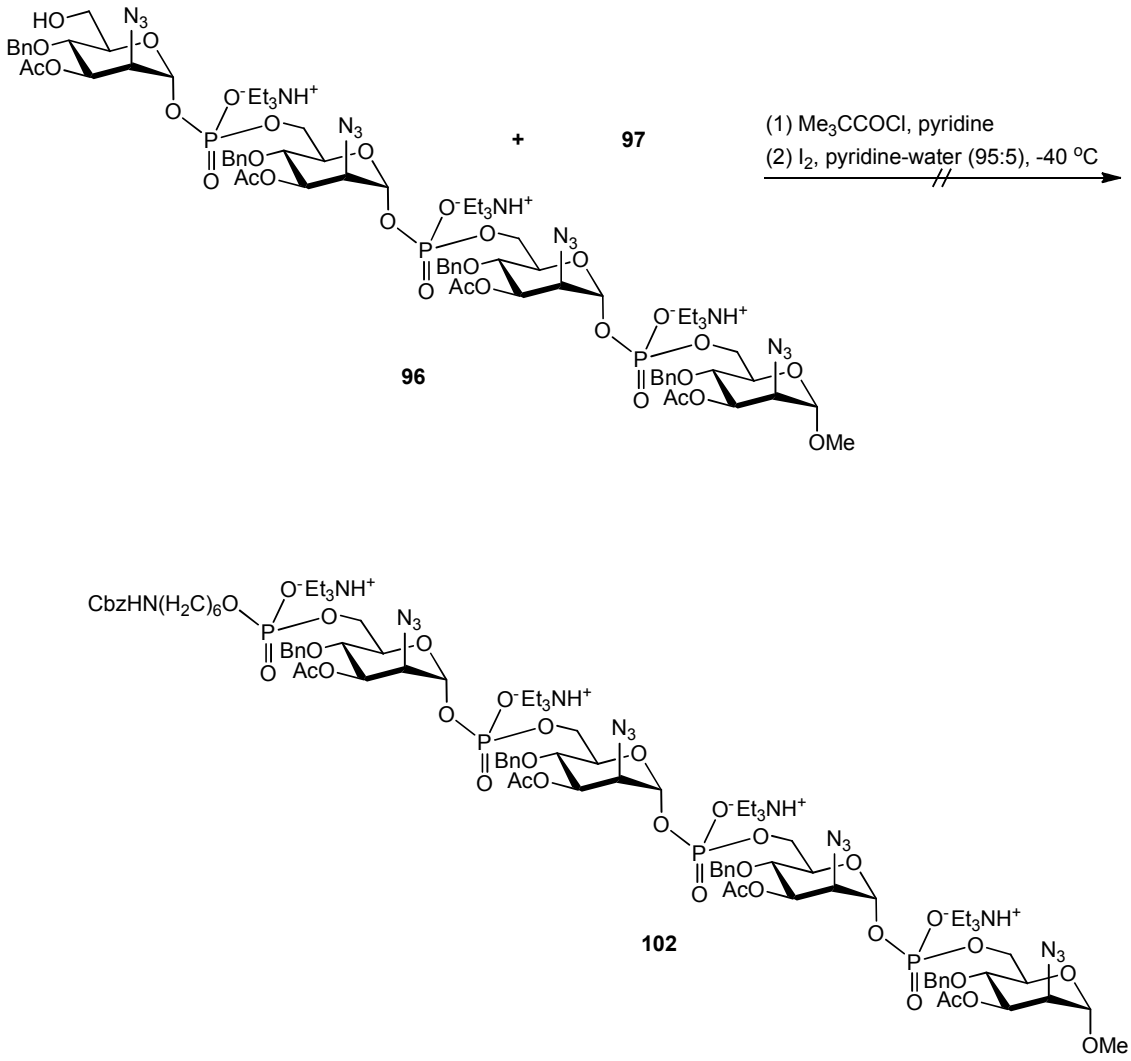


Scheme 38: Detritylation of pentasaccharide tetraphosphate **100**.

Regrettably the resulting mixture of pentasaccharide tetraphosphate **101** and tetrasaccharide triphosphate **96** appeared to be also inseparable on silica gel.

2.6.7 Attempted synthesis of a longer linker containing phosphosaccharide derivative 102.

Following the incomplete synthesis of the pentasaccharide tetraphosphate **100** attention turned to the preparation of the third synthetic target, a linker containing tetrasaccharide tetraphosphate **102**(**Scheme 39**). The tetrasaccharide triphosphate **96** was combined with the H-phosphonate **97** in pyridine before addition of pivaloyl chloride to initiate the condensation.

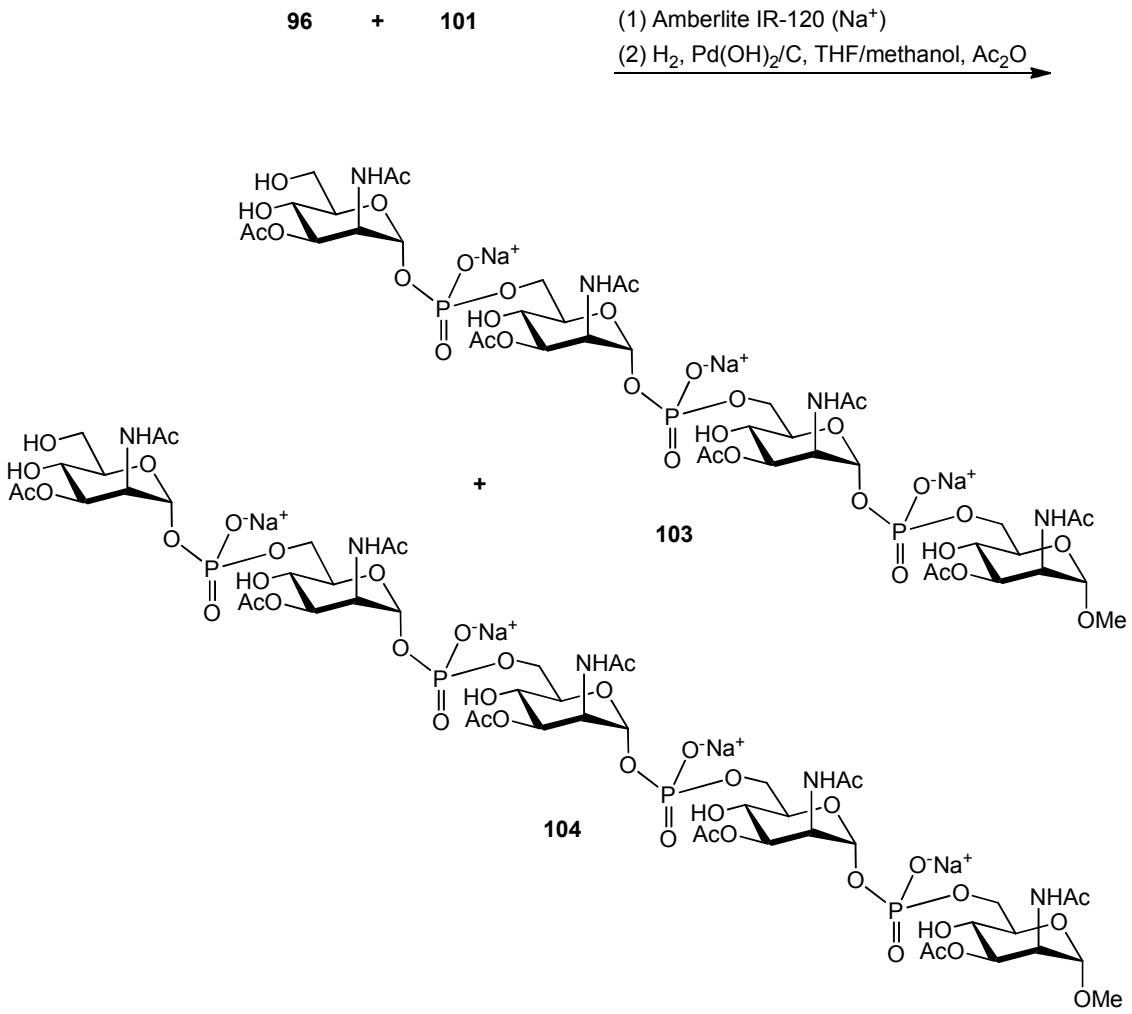


Scheme 39: Attempted formation of a linker containing tetrasaccharide tetraphosphate **102**.

As mentioned earlier monitoring of the reaction using TLC proved difficult. Following coupling, the reaction was cooled to -40 ^{o}C after which time a solution of pyridine/water and Et_3N was added. The oxidation was then initiated with the addition of solid iodine. After purification of what was assumed to be the condensation product**102,**NMR analysis showed very low incorporation of the linker H-phosphonate derivative **97** similar to the attempted preparation of the pentasaccharide tetraphosphate **100**. As the glycosyl H-phosphonate **89** and the linker H-phosphonate **97**had been shown before to react readily in previous syntheses this probably indicates a quite low reactivity of the 6’’’-OH group in the tetrasaccharide triphosphate **96**.

2.6.8Deprotection of a mixture of phosphosaccharide derivatives 96 and 101.

Even though the synthesis of the pentasaccharide tetraphosphate **101**was somewhat unsuccessful and led to an inseparable mixture of pentasaccharide tetraphosphate **101** and tetrasaccharide triphosphate **96** it was still worthy of deprotection. Given that neither of the phosphoglycans contained the amine linker a more straightforward deprotection strategy was utilised (**Scheme 40**). This involved the reduction of the azido functionality to the required amino group and removal of the benzyl ethers through catalytic hydrogenation. Again the triethylammonium salts of the phosphates were exchanged to the sodium salts by treating with IR-120 (Na^+) ion exchange resin. The hydrogenation was then performed over palladium hydroxide on charcoal ($Pd(OH)_2/C$) in a solution of methanol/THF in the presence of acetic anhydride to facilitate *in situ* N-acetylation in a one pot reaction. This led to a mixture of the fully deprotected tetrasaccharide triphosphate **103** andpentasaccharide tetraphosphate **104**.



Scheme 40: Catalytic hydrogenation of a mixture of **96** and **101**.

2.7 CONCLUSIONS

The ultimate aim of this work was to design a synthetic strategy for the preparation of (1-6)-linked poly(2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate) derivatives containing a 3-O acetate group and a linker moiety. To achieve this four different orthogonally protected monosaccharide synthons were prepared (derivatives **56**-**59**) to attempt to find the optimal protecting group arrangement.

The initial synthetic strategy utilised synthons **56** and **57**, derivative **57** (a thioglycoside) being used unsuccessfully as a glycosyl donor in the preliminary glycosylation with 9-decen-1-ol, while derivative **56** was converted to the glycosyl H-phosphonate **77**. The required 9-decen-1-yl glycoside **74** was subsequently prepared starting from the tetra-

acetate derivative **64** via the preparation of the trichloroacetimidate donor **72** followed by a glycosylation with 9-decen-1-ol.

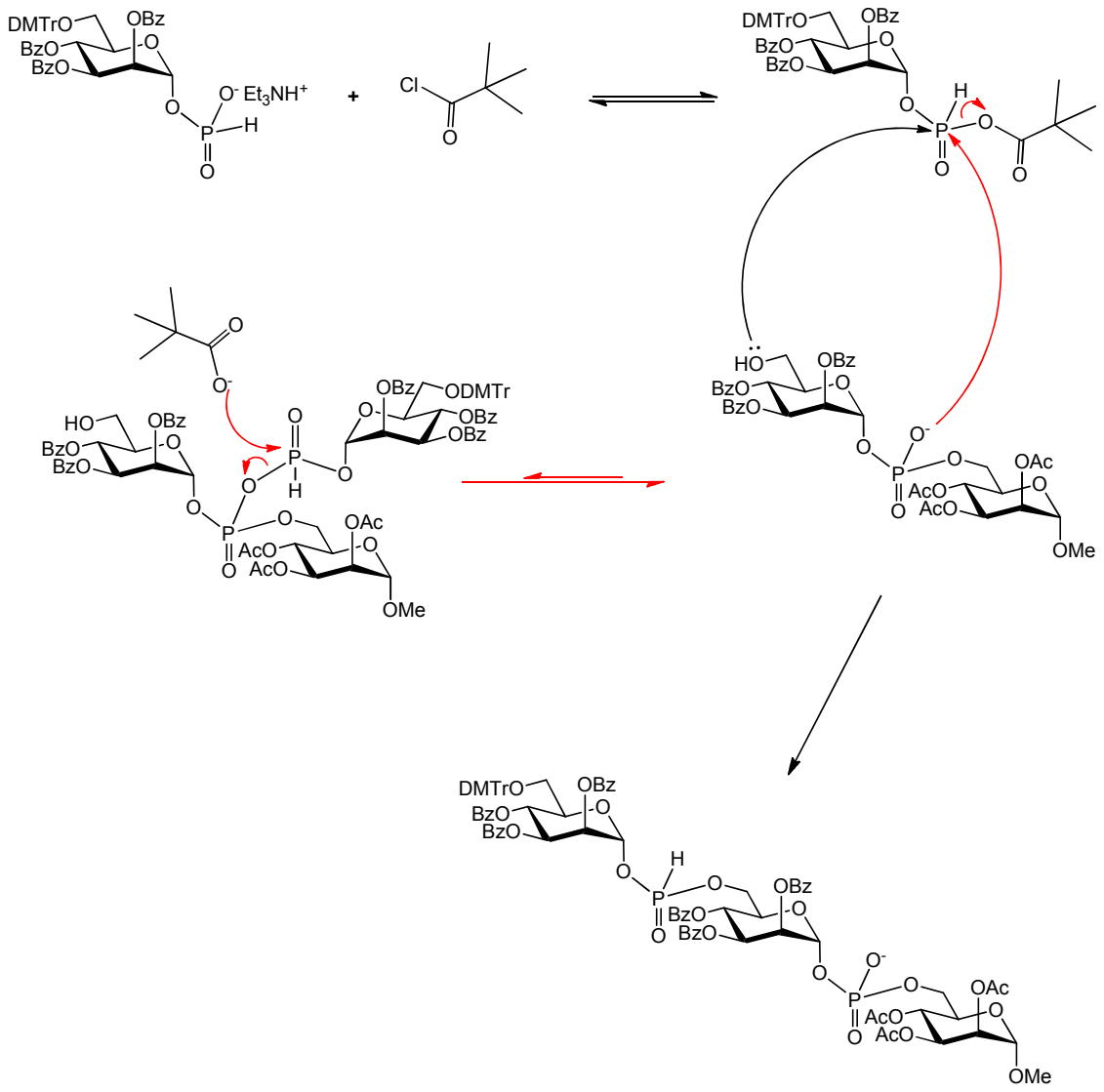
The chemical synthesis of the 9-decenyl linked disaccharide phosphate **79** and the 9-decenyl linked trisaccharide diphosphate **81** was accomplished through the condensation of the DMTr protected H-phosphonate derivative **77** and the monohydroxylic derivatives **78** and **80** respectively. Unfortunately however, during the reduction required for deprotection the 9-decen-yl linker was reduced to a*n*-decanol moiety thus rendering the linker ineffective and so forced a redesigned synthetic strategy.

With the improved synthetic strategy the position of the linker (6-aminohexanol) was moved to the non-reducing end of the phosphoglycan chain and a simpler methyl mannoside (derivative **87**) used as the primary saccharide unit. The O-4 protection of the primary saccharide unit **87** was a benzyl ether and so to keep the protecting group arrangement consistent this approach concentrated on the monosaccharide synthons **58** and **59**, both of which also contain the O-4 benzyl protection. Both synthons **58** and **59** were successfully converted to the required H-phosphonate derivatives **87** and **89** through the use of the phosphonylating agent salicylchlorophosphite and assessed for condensation efficiency.

The chain elongation was successful in the integration of up to 3 intersaccharidic phosphates in a phosphoglycan molecule (compounds **95** and **96**). Also the introduction of a fourth intersaccharidic phosphate was in partial success, but the reaction did not go to completion and resulted in an inseparable mixture of pentasaccharide tetraphosphate **101** and tetrasaccharide triphosphate **96**.

Some interesting observations have been made in terms of the condensation reaction time required for the preparation of the disaccharide phosphate **92** (coupling the

glycosyl H-phosphonate **89** and the monosaccharide derivative **87**), trisaccharide diphosphate **94** (coupling the glycosyl H-phosphonate **89** and the disaccharide monophosphate derivative **92**) and the tetrasaccharide triphosphate **95** (coupling the glycosyl H-phosphonate **89** and the trisaccharide diphosphate derivative **94**).In the first condensation with the glycosyl H-phosphonate **89** and the monosaccharide derivative **87**,the condensation was complete (as monitored by TLC) after only 5 minutes after the addition of pivaloyl chloride. For the second condensation with the glycosyl H-phosphonate **89** and the disaccharide monophosphate derivative **92**the reaction time increased to around 20 minutes after the addition of pivaloyl chloride. With the introduction of the third intersaccharidic phosphate, the condensation between the glycosyl H-phosphonate **89** and the trisaccharide diphosphate derivative **94**the reaction time increased further to around 40 minutes after initiation with pivaloyl chloride. These observations are in line with the proposed reaction mechanism shown by Nikolaev and co-workers in 1992 while investigating the suitability of the H-phosphonate approach in the synthesis of oligo(mannosyl phosphate) derivatives.[116] An example of this is shown in **Scheme 41**. During the condensation, with a monohydroxylic derivative, which already contains a phosphate group, both the hydroxyl and the oxygen of the phosphate can act as the nucleophile. Nucleophilic attack from the hydroxyl group leads to the required H-phosphonic diester in an irreversible reaction. The latter scenario, nucleophilic attack from the phosphate, results in an unstable mixed anhydride [i.e., glycosyl H-phosphono(phosphoric) anhydride] being formed in a reversible reaction with the equilibrium in favour of the starting materials.[92] This goes some way in explaining why the presence of a phosphate group slows the reaction time of the condensation and the reaction time increases further with two phosphate groups present.



Scheme 41: Proposed H-phosphonate reaction mechanism.

Introduction of the linker H-phosphonate **97** into phosphosaccharides **92** and **94**was achieved without issue to give the linker containing disaccharide diphosphate **98** andthe linker containing trisaccharide triphosphate **99**. However, the introduction of the linker H-phosphonate **97** into the tetrasaccharide triphosphate **96** was unsuccessful due to reasons unclear at this time.

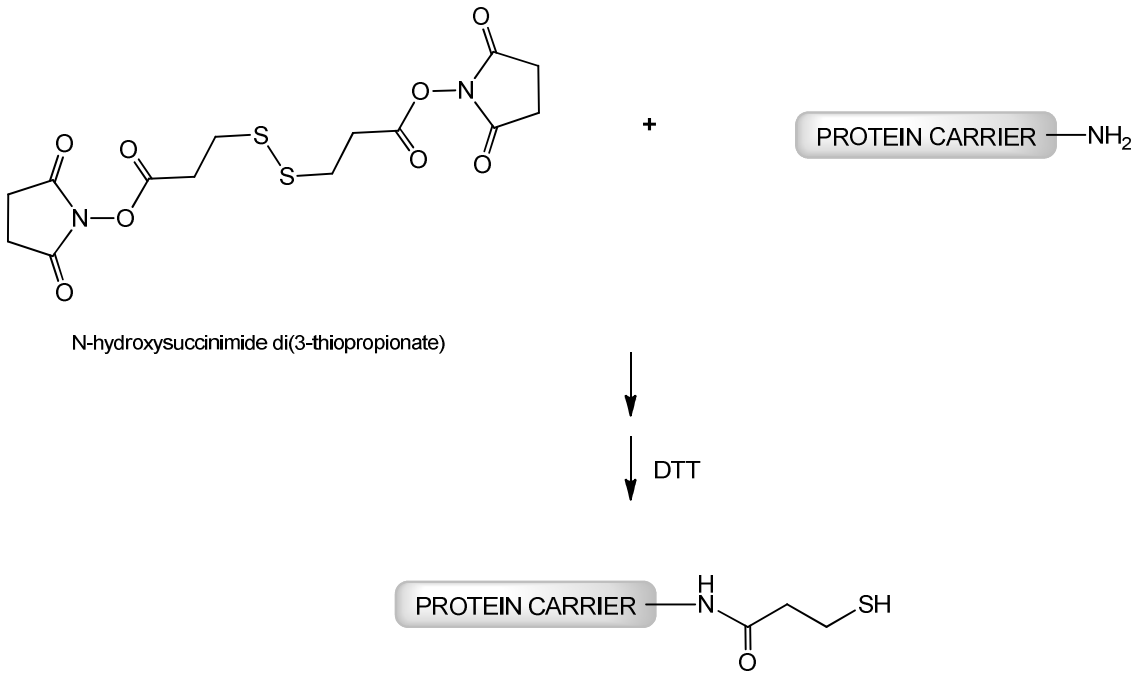
Following deprotection the desired linker containing synthetic targets, the disaccharide diphosphate**83** and the trisaccharide triphosphate **84** were obtained in high yield complete with a linker containing an amine functionality allowing for bioconjugation.

2.8 SUITABLE BIOCONJUGATION TECHNIQUES FOR THE PREPARED PHOSPHOGLYCANS

2.8.1 Suitable bioconjugation techniques

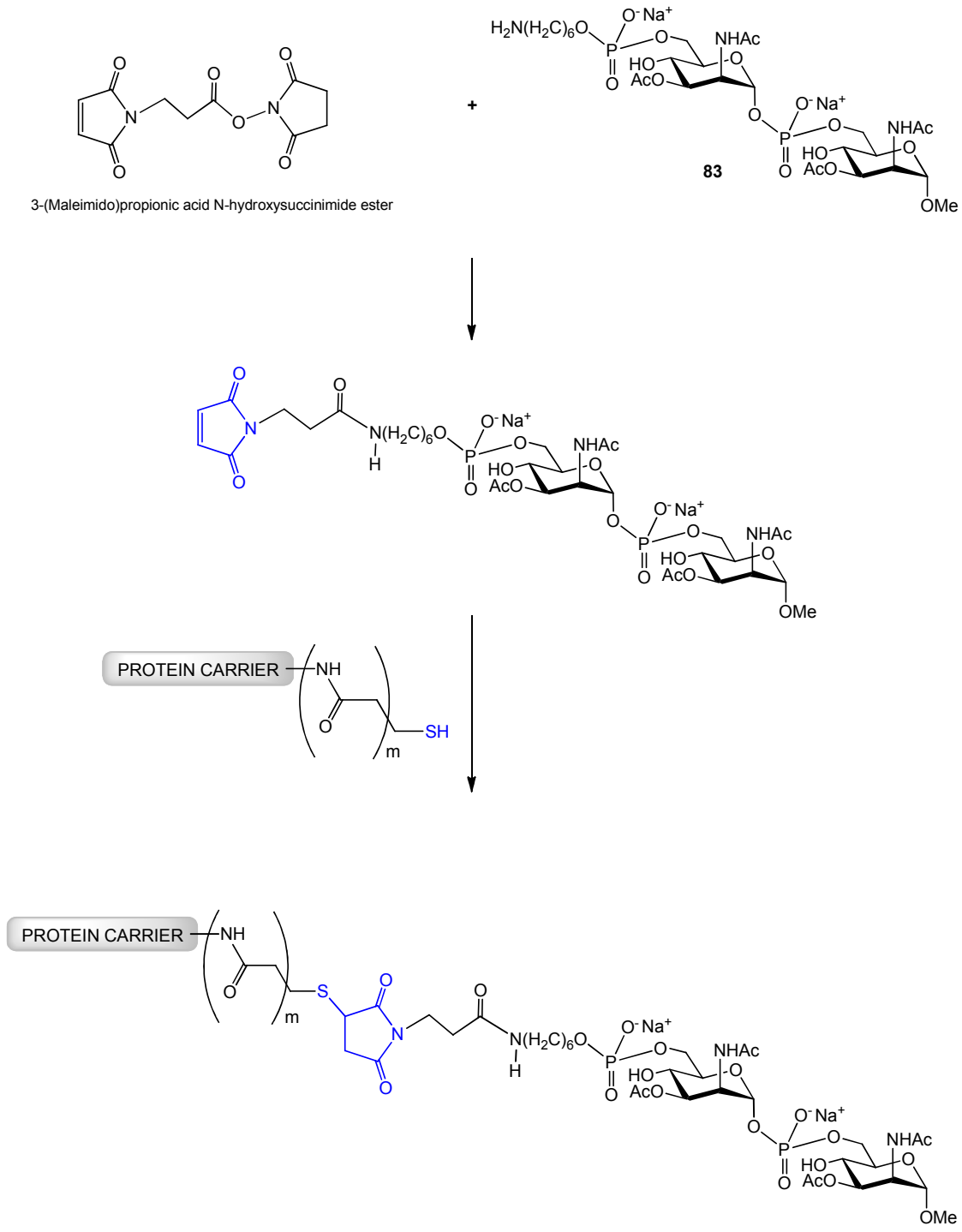
Although, due to time constraints, no bioconjugation of the synthesised CPS fragments **83** and **84**was performed it is still worth mentioning conjugation techniques which would be suitable for our structures. These methods were chosen for two reasons. The first was simply that they both require an amine functionality in the linker andsecondly both have been shown to work well in the preparation of glycoconjugates where the polysaccharide used was a phosphoglycan containing fragile intersaccharidic phosphodiester linkages.

The first possible method was used by Verez-Bencomo and co-workers in 2004 in the preparation of the synthetic conjugate vaccine against *Haemophilus influenza* type b[86]which is composed of ribosylribitol phosphate repeating units (see **Figure 7**, section **1.1.6**). In this approach the desired protein is first treated with bis-succinimidyl di(3-thiopropionate) (**Scheme 42**).[117]



Scheme 42: Protein modification using N-hydroxysuccinimide di(3-thiopropionate).

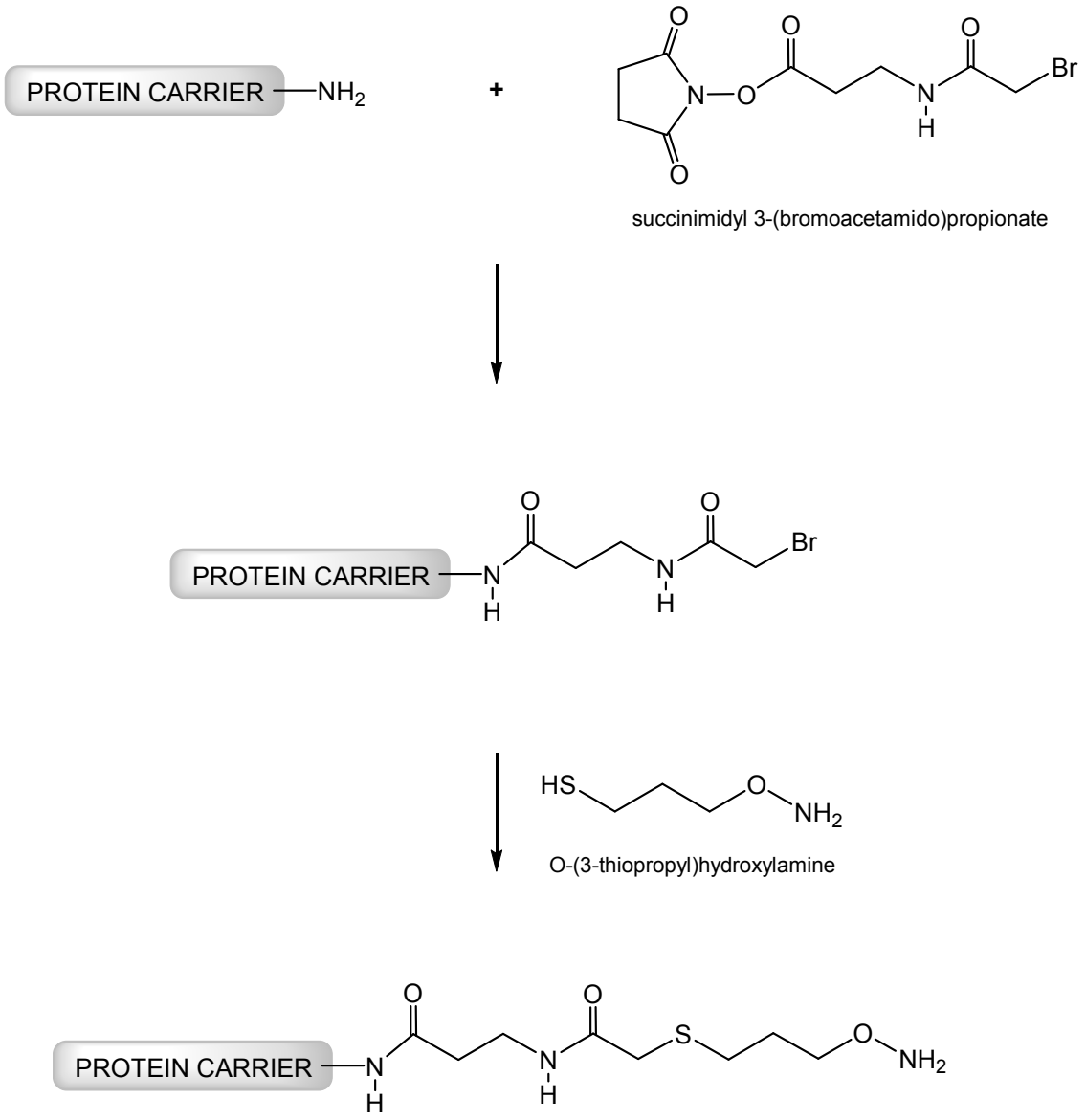
Following addition treatment with dithiothreitol (DTT) gives the required thiolated protein suitable for conjugation. The linker containing phosphoglycans (**83** or **84**) must also be modified with 3-(maleimido)propionic acid N-hydroxysuccinimide ester (**Scheme 43**).



Scheme 43: Phosphoglycan modification and protein conjugation.

The resulting 3-(maleimido)propionamido phosphoglycan derivative can then be conjugated to the thiolated protein resulting in a glycoconjugate suitable for antigenicity trials.

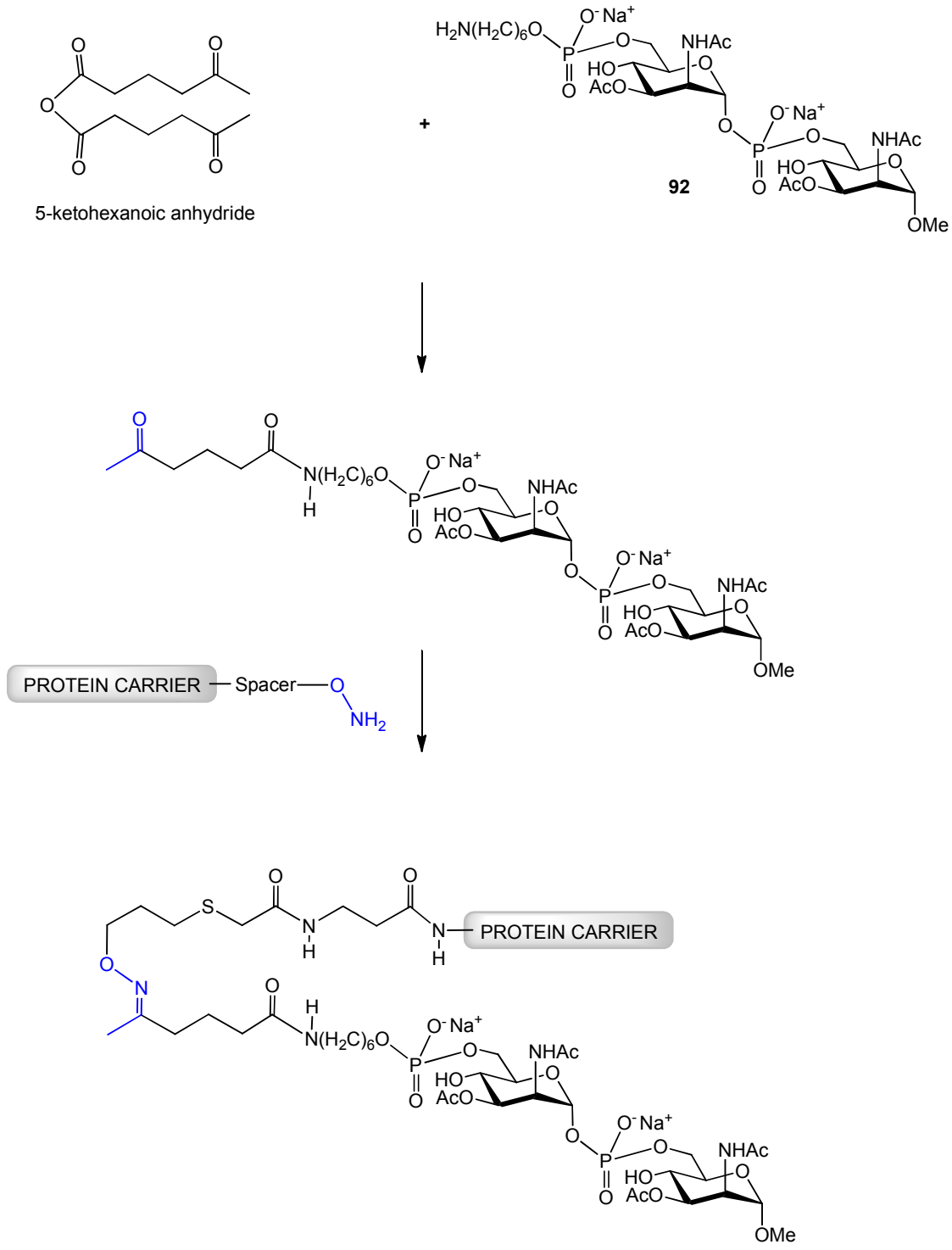
Another suitable method was developed by Pozsgay and Kubler-Kielb in 2005.[118] It is based on the known oxime formation between a protein modified with O-alkyl hydroxylamine grouping and a phosphoglycan modified with a carbonyl group containing spacer.



Scheme 44: Protein modification using succinimidyl 3-(bromoacetamido)propionateand O-(3-thiopropyl)hydroxylamine.

To this end, the chosen protein is first treated with succinimidyl 3-(bromoacetamido)propionate(**Scheme 44**). The resulting bromoacylated protein then

reacted with O-(3-thiopropyl)hydroxylamine, thus making corresponding aminooxylated protein derivative.



Scheme 45: AlternativePhosphoglycan modification and protein conjugation.

With subsequent treatment of the linker containing phosphoglycan (**83** or **84**) with 5-ketohexanoic anhydride the conjugation of the prepared 5-ketohexanoyl phosphoglycan derivative to the previously modified aminooxylated protein can be performed (**Scheme 45**). This again would hopefully provide glycoconjugates suitable for further testing in antigenicity trials.

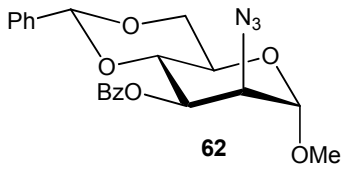
CHAPTER 3

3. EXPERIMENTAL

General Procedures

Melting points were determined on a Griffin melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 ^{o}C; $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded with Bruker AVANCE 500 MHz spectrometer, unless otherwise indicated. Chemical shifts (δ in ppm)are given relative to those for Me_4Si (for ^{1}H and ^{13}C) and external aq. 85% H_3PO_4 (for ^{31}P); all *J* values are given in Hz. ES mass spectra were recorded with a Mariner™ Biospectrometry Workstation and Aglient spectrometer. High resolution mass spectra were recorded on a Bruker microTOF. IR spectra were recorded with Nicolet 205 FT-IR spectrometer. Thin layer chromatography (TLC) was performed on silica gel60 F_{254} aluminium plates (Merck) with various solvent systems as developers, followed by detection under UV light and/or by charring, which was carried out with sulphuric acid-water-ethanol (15:85:5) for protected compounds or a mixture of orcinol containing sulphuric acid-ethanol-water (10:75:5) for final deprotected compounds. Flash columnchromatography (FCC) was performed on Kieselgel 60 (0.040-0.063 mm) (Merck), unless otherwise indicated. Dichloromethane, acetonitrile, pyridine and toluene were freshly distilled from CaH_2. Petroleum ether refers to that with boiling fraction 40-60 ^{o}C, unless otherwise stated. Solutions worked up were concentrated under reduced pressure at < 40 ^{o}C.

Methyl 2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside 62



Triflic anhydride (26.3 ml, 159.5 mmol, 1.8 eq.) was added to a cooled (-30 °C) solution of the diol **60** (25 g, 88.6 mmol) in a mixture of CH_2Cl_2 (100 ml) and pyridine (40 ml). The solution was stirred at -30 °C for 2 h, after which benzoyl chloride (14.4 ml, 124 mmol, 1.4 eq.) was added. Cooling was then discontinued and after warming to room temperature the reaction mixture was quenched with saturated aq. $NaHCO_3$ before extracting with CH_2Cl_2. The organic extract was then washed successively with water, 1 M HCl, water, and brine and finally dried (Na_2SO_4) before concentrating under reduced pressure. The residue (crude 2-triflate) was then dissolved in DMF (150 ml) and NaN_3 (37.3 g, 573 mmol, 6.5 eq) was added. The mixture was then heated to 70 °C with stirring for 16 h. The reaction mixture was then diluted with EtOAc before washing with water, brine and dried (Na_2SO_4), before concentrating under reduced pressure. FCC [toluene-EtOAc, (95:5)] of the residue gave the azido derivative **62** (26.60 g, 73%, 64.68 mmol,) as an amorphous solid.

υ_{max} (film)/cm-1 (*inter alia*): 1723 (C=O), 2104 (-N_3);

$[\alpha]_D$ +63.1 (*c* 1, $CHCl_3$);

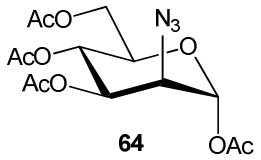
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 3.37 (3H, s, OCH_3), 3.81 (1H, t, $J_{5,6a} = J_{6a,6b}$ = 10.2, H-6a), 3.91 (1H, ddd, $J_{5,6b}$ 4.6, H-5), 4.15 (1H, t, $J_{3,4} = J_{4,5}$ = 10.0, H-4), 4.20 (1H, dd, $J_{2,3}$ 3.9, H-2), 4.23 (1H, dd, $J_{5,6b}$ 4.6, H-6b), 4.69 (1H, d, $J_{1,2}$ 1.5, H-1), 5.54 (1H, s, PhC*H*), 5.67 (1H, dd, $J_{2,3}$ 3.9, H-3) and 7.24-8.05 (10H, m, 2 x Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 55.25 (OCH_3), 62.44 (C-2), 63.92 (C-5), 68.73 (C-6), 70.47 (C-3), 76.30 (C-4), 100.04 (C-1), 101.89 (Ph*C*H), 126.13-137.04 (Ph) and 165.59 (C=O).

ES-MS(+): found *m/z* 434.13 $[M+Na]^+$ ($C_{21}H_{21}N_3O_6$requires M, 411.1430).

High resolution ES-MS(+): found *m/z* 412.1498 $[M+H]^+$ ($C_{21}H_{22}N_3O_6^+$ requires *m/z*, 412.1503), 429.1760 $[M+NH_3+H]^+$ ($C_{21}H_{25}N_4O_6^+$ requires *m/z*, 429.1774); ($C_{21}H_{21}N_3O_6$ requires M, 411.1430).

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose 64



64

A solution of 4.5 M sodium methoxide in methanol (1.00 ml) was added to a stirring solution of compound **62** (3 g, 7.29 mmol) in a mixture of CH_2Cl_2 (30 ml) and methanol (30 ml). The solution was then stirred at room temperature for 1 h after which it was deionised with the addition of DOWEX 50WX4-50 (H^+) resin. The resin was then filtered off and the filtrate was concentrated under reduced pressure. The residue was co-evaporated with toluene (3 x 50 ml) and then dissolved in acetic anhydride (30 ml) and cooled to 0 °C, before the addition of 2% (v/v) H_2SO_4 in acetic anhydride (600 µl). The solution was maintained at 0 °C with stirring for 4 h, then allowed to warm to room temperature and stirred for an additional 1 h. Ice was added to quench the reaction. The mixture was allowed to stir for 1 h and then extracted with CH_2Cl_2. The organic layer was washed with water, saturated aq. $NaHCO_3$, and dried (Na_2SO_4) before

concentrating under reduced pressure. FCC [toluene-EtOAc, (9:1)] of the residue gave the tetra-acetate derivative **64** (2.21 g, 81%) as an amorphous solid.

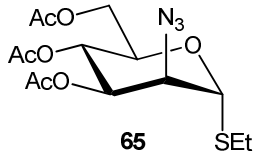
υ_{max} (film)/cm^{-1} (*inter alia*): 1724 (C=O), 2102 (-N_3);

$[\alpha]_D$ +86.7 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.99, 2.04, 2.06 and 2.10 (12H, 4 x s, 4 x Ac), 3.95 (1H, ddd, $J_{5,6a}$ 2.3, H-5), 3.97 (1H, dd, $J_{2,3}$ 3.4, H-2), 4.03 (1H, dd, $J_{6a,6b}$ 12.5, H-6a), 4.19 (1H, dd, $J_{5,6b}$ 4.5, H-6b), 5.29-5.36 (2H, m, H-3 and H-4) and 6.05 (1H, d, $J_{1,2}$ 1.9, H-1);
^{13}C NMR (125 MHz; $CDCl_3$): δ_C 20.57, 20.67, 20.78 and 20.95 (CH_3CO), 60.52 (C-2), 61.77 (C-6), 65.29 (C-4), 70.58 and 70.76 (C-3 and C-5), 91.38 (C-1) and 168.26, 169.42, 170.13 and 170.79 (CH_3C=O).

ES-MS(+): found *m/z* 396.10 $[M+Na]^+$ ($C_{14}H_{19}N_3O_9$ requires M, 373.1121).
High resolution ES-MS(+): found *m/z* 391.1471 $[M+NH_3+H]^+$ ($C_{14}H_{23}N_4O_9^+$ requires *m/z*, 391.1465), 396.1029 $[M+Na]^+$ ($C_{14}H_{19}N_3NaO_9^+$ requires *m/z*, 396.1019), 769.2146 $[2M+Na]^+$ ($C_{28}H_{38}N_6NaO_{18}^+$ requires *m/z*, 769.2140); ($C_{14}H_{19}N_3O_9$ requires M, 373.1121).

Ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-mannopyranoside 65



Ethanethiol (405 µl, 5.46 mmol, 2 eq.) and $BF_3{\cdot}Et_2O$ (1.38 ml, 10.92 mmol, 4 eq.) were

added to a solution of the tetra-acetate **64** (1.02 g, 2.73 mmol) in CH_2Cl_2 (10 ml) prior to the addition of freshly activated 4Å molecular sieves (1 g). The solution was then stirred under argon for 16 h before quenching the reaction with the addition of Et_3N (2.5 ml, 17.8 mmol). The reaction mixture was then applied directly onto a SiO_2 column and flushed with toluene to remove the excess ethanethiol. The product was then eluted from the column with toluene-EtOAc (1:1) and corresponding fractions were combined and concentrated under reduced pressure. FCC [toluene-EtOAc, (9:1)] of the residue gave the thioglycoside derivative **65** (938 mg, 91%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1722 (C=O), 2101 (-N_3);

$[\alpha]_D$ +91.4 (*c* 1, $CHCl_3$);

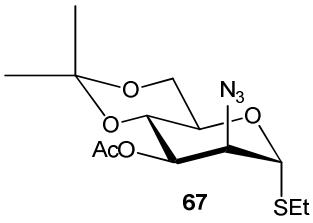
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.25 (3H, t, *J* 7.4, CH_2CH_3), 1.99, 2.03 and 2.04 (9H, 3 x s, 3 x Ac), 2.51-2.65 (2H, m, SCH_2), 4.02 (1H, dd, $J_{6a,6b}$ 12.3, H-6a), 4.06 (1H, dd, $J_{2,3}$ 3.6, H-2), 4.22 (1H, dd, $J_{5,6b}$ 4.9, H-6b), 4.29 (1H, ddd, $J_{5,6a}$ 2.3, H-5), 5.23 (1H, dd, $J_{2,3}$ 3.6, H-3), 5.27 (1H, d, $J_{1,2}$ 1.4, H-1) and 5.28 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, H-4);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.70 (CH_3CH_2), 20.55, 20.67 and 20.73 (CH_3CO), 25.44 (SCH_2), 62.12 (C-6), 62.79 (C-2), 66.06 (C-4), 68.70 (C-5), 71.32 (C-3), 82.32 (C-1) and 169.57, 169.91 and 170.70 (CH_3C=O).

ES-MS(+): found *m/z* 398.10 $[M+Na]^+$ ($C_{14}H_{21}N_3O_7S$ requires M, 375.1100).

High resolution ES-MS(+): found *m/z* 393.1448 $[M+NH_3+H]^+$ ($C_{14}H_{25}N_4O_7S^+$ requires *m/z*, 393.1444), 398.1004 $[M+Na]^+$ ($C_{14}H_{21}N_3NaO_7S^+$ requires *m/z*, 398.0998),773.2100 $[2M+Na]^+$ ($C_{28}H_{42}N_6NaO_{14}S_2^+$ requires *m/z*, 773.2098); ($C_{14}H_{21}N_3O_7S$ requires M, 375.1100).

Ethyl 3-*O*-acetyl-2-azido-2-deoxy-4,6-*O*-isopropylidene-1-thio-α-D-mannopyranoside 67



A solution of 4.5 M sodium methoxide in methanol (250 μl) was added to a stirring solution of the thioglycoside **65** (567 mg, 1.51 mmol) in a mixture of CH_2Cl_2 (10 ml) and methanol (10 ml). The solution was then stirred at room temperature for 1h before deionising with the addition of DOWEX 50WX4-50 (H^+) resin. The resin was then filtered off and the filtrate was concentrated under reduced pressure. The residue was co-evaporated with toluene (3 x 20 ml) and then dissolved in a mixture of DMF (4 ml) and dry acetone (0.8 ml). 2,2-Dimethoxypropane (1.2 ml) and *p*-toluenesulphonic acid (15 mg) were then added and the solution was stirred at room temperature for 5 h, prior acetic anhydride (1.2 ml) and pyridine (4 ml) were added and stirring was continued for another 16 h. Water was added and the mixture was extracted with EtOAc. The organic layer was washed successively with 1 M HCl, water and saturated aq. $NaHCO_3$ and dried (Na_2SO_4) before concentrating under reduced pressure. FCC [toluene-EtOAc, (19:1)] of the residue gave the derivative **67** (400 mg, 80%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1718 (C=O), 2105 (-N_3);

$[\alpha]_D$ +73.6 (*c* 1, $CHCl_3$);

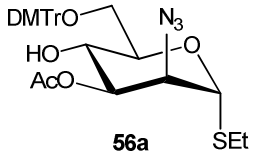
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.29 (3H, t, *J* 6.4, SCH_2CH_3), 1.41, (3H, s, CCH_3), 1.52 (3H, s, CCH_3), 2.14 (3H, s, Ac), 2.62 (2H, m, SCH_2CH_3), 3.79-3.86 (2H, m, H-6^a +

H-6^{b}), 4.08 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, H-4), 4.13 (1H, m, H-5), 4.21 (1H, dd, $J_{2,3}$ 3.8, H-2), 5.19 (1H, dd, $J_{2,3}$ 3.8, H-3) and 5.25 (1H, d, $J_{1,2}$ 1.1, H-1);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.74 (*C*H_3CH$_2$), 19.21 (*C*H_3C), 20.74 (*C*H_3CO), 25.36 (CH$_3$*C*H$_2$), 29.10 (*C*H$_3$C), 61.96 (C-6), 63.38 (C-2), 65.29 (C-5), 69.00 (C-4), 70.82 (C-3), 83.09 (C-1), 100.17 (*C*(CH$_3$)$_2$) and 170.13 (CH$_3$*C*=O).

ES-MS(+): found *m/z* 354.11 $[M+Na]^+$ ($C_{13}H_{21}N_3O_5S$ requires M, 331.12).

Ethyl 3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-1-thio-α-D-mannopyranoside 56a

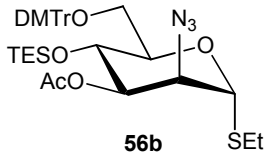


56a

Trifluoroacetic acid (1.5 ml) and water (50 μl) was added to a stirred solution of compound **67** (160 mg, 0.48 mmol) in CH_2Cl_2 (15 ml). The mixture was stirred at room temperature for 45 min before concentrating under reduced pressure, followed by co-evaporation with toluene. The residue was then dissolved in pyridine (1.5 ml) followed by the addition of *p,p'*-dimethoxytrityl chloride (204 mg, 0.60 mmol, 1.25 eq.). The solution was stirred for 16 h before water was added and the mixture was diluted with CH_2Cl_2. The organic layer was then washed with water, dried (Na_2SO_4) and concentrated before co-evaporation with toluene. FCC [toluene-EtOAc, (19:1)→(4:1)] of the residue gave the DMTr derivative **56a** (260 mg, 91%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.29 (3H, t, *J* 6.4, CH_2CH_3), 2.15, (3H, s, Ac), 2.62 (2H, m, CH_2CH_3), 2.69 (1H, d, $J_{4,\ 4\text{-OH}}$ 3.45, 4-OH), 3.42 (2H, d, $J_{5,6}$5.10, H-6), 3.79 (6H, s, 2 x OMe), 3.97 (1H, dt, $J_{3,4} = J_{4,5} = 9.6$, H-4), 4.09 (1H, dd, $J_{1,2}$ 1.4, $J_{2,3}$ 3.6, H-2), 4.12 (1H, dt, $J_{5,6}$5.10, H-5), 5.16 (1H, dd, $J_{3,4}$ 9.6, H-3) and 5.27 (1H, d, $J_{1,2}$ 1.4, H-1).

Ethyl 3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-1-thio-α-D-mannopyranoside 56b



56b

Triethylsilyl triflate (113 μl, 0.50 mmol, 1.25 eq.) was added to a cooled (0 °C) solution of compound **56a** (238 mg, 0.40 mmol) in a mixture of pyridine (1.5 ml) and Et_3N (0.4 ml). The solution was stirred at 0 °C for 15 min and diluted with water before extraction with CH_2Cl_2. The organic layer was then washed with saturated aq. $NaHCO_3$, before concentrating under reduced pressure. Toluene was evaporated off from the residue. FCC [toluene-EtOAc, (19:1)] of the residue gave the derivative **56b** (243 mg, 86%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1725 (C=O), 2109 (-N_3);

$[\alpha]_D$ +58.7 (*c* 1, $CHCl_3$);

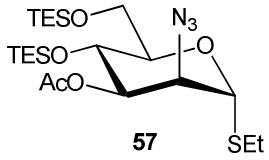
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 0.33 (6H, q, *J* 8.0, Si(CH_2CH_3)$_3$), 0.75 (9H, t, *J* 8.0, Si(CH_2CH_3)$_3$) 1.21 (3H, t, *J* 7.4, SCH_2CH_3), 1.94 (3H, s, Ac), 2.53-2.70 (2H, m,

S*CH*$_2$CH$_3$), 3.02 (1H, dd, $J_{5,6a}$ 7.1, $J_{6a,6b}$ 9.7, H-6^a), 3.15 (1H, dd, $J_{5,6b}$ 1.8, H-6^b), 3.60 (6H, s, 2 x OMe), 3.69 (1H, t, $J_{3,4} = J_{4,5} = 9.3$, H-4), 3.97 (1H, dd, $J_{1,2}$ 1.4, $J_{2,3}$ 3.8, H-2), 4.07 (1H, ddd, $J_{4,5}$ 9.3,H-5), 4.89 (1H, dd, $J_{2,3}$ 3.8, $J_{3,4}$ 9.3, H-3), 5.21 (1H, d, $J_{1,2}$ 1.4, H-1) and 6.61-7.30 (13H, m, Ph, 2 x C_6H_4);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 4.86 (CH_3CH_2Si), 6.82 (CH_3CH_2Si), 14.54 (CH_3CH_2S), 20.87 (CH_3CO), 24.74 (CH_3CH_2S), 55.22 (CH_3O), 62.57 (C-2), 63.25 (C-6), 66.95 (C-4), 73.24 (C-5), 74.98 (C-3), 81.05 (C-1), 85.90 (Ar_3C), 113.02-158.44 (C_6H_4, Ph) and 170.08 (CH_3C=O).

ES-MS(+): found *m/z* 730.30 $[M+Na]^+$ ($C_{37}H_{49}N_3O_7SSi$ requires M, 707.31).

Ethyl 3-*O*-acetyl-2-azido-2-deoxy-4,6-di-*O*-triethylsilyl-1-thio-α-D-mannopyranoside 57



Trifluoroacetic acid (1.5 ml) and water (50 μl) was added to a stirred solution of compound **67** (169 mg, 0.51 mmol) in CH_2Cl_2 (15 ml). The solution was stirred at room temperature for 45 min before concentrating under reduced pressure, followed by co-evaporation with toluene. The residue was then dissolved in a mixture of pyridine (2 ml) and Et_3N (0.53 ml), the solution was then cooled (0 °C) prior the addition of triethylsilyl triflate (288 μl, 1.28 mmol, 2.5 eq.). The solution was stirred at 0 °C for 15 min, quenched with water before extraction with CH_2Cl_2. The organic layer was then washed

with saturated aq. $NaHCO_3$ before concentrating under reduced pressure. Toluene was evaporated off from the residue. FCC [toluene-EtOAc, (19:1)] of the residue gave the derivative **57** (230 mg, 87%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1726 (C=O), 2102 ($-N_3$);

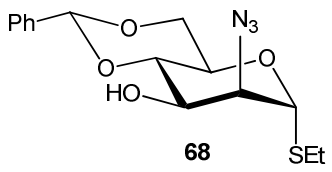
$[\alpha]_D$ +49.6 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 0.51-0.59 (12H, m, 2 x Si(CH_2CH_3)$_3$), 0.87-0.91 (18H, m, 2 x Si(CH_2CH_3)$_3$) 1.21 (3H, t, *J* 7.4, SCH_2CH_3), 2.08 (3H, s, Ac), 2.47-2.64 (2H, m, SCH_2CH_3), 3.71 (1H, dd, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 11.2, H-6^a), 3.76 (1H, dd, $J_{5,6b}$2.0, H-6^b), 3.90 (1H, ddd, $J_{5,6a}$ 5.1, $J_{5,6b}$2.0, H-5), 3.95 (1H, t, $J_{3,4} = J_{4,5}$ = 9.2, H-4), 4.04 (1H, dd, $J_{2,3}$ 3.8, H-2), 4.99 (1H, dd, $J_{2,3}$ 3.8, H-3) and 5.2 (1H, d, $J_{1,2}$ 1.3, H-1);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 4.48 (CH_3CH_2Si), 5.05 (CH_3CH_2Si), 6.72 (CH_3CH_2Si), 6.83 (CH_3CH_2Si), 14.60 (CH_3CH_2S), 20.92 (CH_3CO), 24.91 (CH_3CH_2S), 61.94 (C-6), 62.62 (C-2), 66.62 (C-4), 74.33 (C-5), 76.78 (C-3), 81.45 (C-1) and 170.12 (CH_3C=O).

ES-MS(+): found *m/z* 542.25 $[M+Na]^+$ ($C_{22}H_{45}N_3O_5SSi_2$ requires M, 519.26).

Ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside 68



68

The thioglycoside **65** (750 mg, 2 mmol) was deacetylated as described for the

preparation of compound 6**7**. To a stirred solution of the prepared ethyl 2-azido-2-deoxy-1-thio-α-D-mannopyranoside (2 mmol) and benzaldehyde dimethylacetal (1.15 ml, 7.49 mmol, 3.75 eq.) in MeCN (20 ml) activated $NaHSO_4{\cdot}SiO_2$ (500 mg) was added. The reaction mixture was stirred at room temperature for 0.5 h and quenched with the addition of Et_3N (0.5 ml). The mixture was then filtered through a Celite pad and the filtrate was concentrated under reduced pressure. FCC [toluene-EtOAc, (9:1)] of the residue gave the benzylidene derivative **68** (572 mg, 85%).

υ_{max} (film)/cm-1 (*inter alia*): 2105 ($-N_3$);

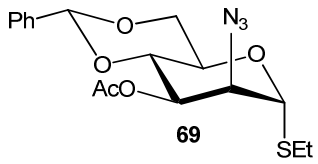
$[\alpha]_D$ +68.9 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.34 (3H, t, *J* 7.4, $SCH_2\mathit{CH}_3$), 2.61 (1H, d, $J_{H,OH}$ 3.9, 3-OH), 2.61-2.75 (2H, m, $S\mathit{CH}_2CH_3$), 3.86 (1H, m, H-6^a), 3.96 (1H, t, $J_{3,4} = J_{4,5} = 9.5$, H-4), 4.13 (1H, dd, $J_{2,3}$ 3.9, H-2), 4.21-4.30 (3H, m, H-4, H-5 and H-6^b), 5.32 (1H, d, $J_{1,2}$ 1.1, H-1) 5.61 (1H, s, PhC*H*) and 7.38-7.59 (5H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.86 ($\mathit{C}H_3CH_2S$), 25.58 ($CH_3\mathit{C}H_2S$), 63.85 (C-5), 65.10 (C-2), 68.48 (C-6), 69.40 (C-3), 79.28 (C-4), 83.30 (C-1), 102.34 (Ph*C*H) and 126.25, 128.42, 129.40, 137.00 (Ph).

ES-MS(+): found *m/z* 360.10 $[M+Na]^+$ ($C_{15}H_{19}N_3O_4S$ requires M, 337.11).

Ethyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside 69



To a stirred solution of compound **68** (570 mg, 1.69 mmol) in pyridine (10 ml) acetic anhydride (5 ml) was added. The reaction mixture was stirred at room temperature for 1.5 h before concentrating under reduced pressure. The residue was then co-evaporated three times with toluene. FCC [toluene-EtOAc, (19:1)] of the residue gave the benzylidene derivative **69** (632 mg, 98%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1720 (C=O), 2103 ($-N_3$);

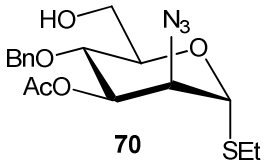
$[\alpha]_D$ +86.7 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.24 (3H, t, *J* 7.4 SCH_2CH_3), 2.06 (1H, s, Ac), 2.51-2.65 (2H, m, SCH_2CH_3), 3.79 (1H, t, $J_{5,6a} = J_{6a,6b} = 10.3$, H-6^a), 4.03 (1H, t, $J_{3,4} = J_{4,5} =$ 9.9, H-4), 4.16 (1H, dd, $J_{5,6b}$ 4.9, H-6^b), 4.19 (1H, dd, $J_{1,2}$ 1.1, H-2), 4.27 (1H, ddd, H-5) and 5.22 (1H, d, H-1) 5.31 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.50 (1H, s, PhC*H*) and 7.25-7.42 (5H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.77 (CH_3CH_2S), 21.20 (CH_3CO), 25.46 (CH_3CH_2S), 63.39 (C-2), 64.34 (C-5), 68.48 (C-6), 70.41 (C-3), 76.31 (C-4), 83.18 (C-1), 102.02 (PhCH) and 126.23, 128.33, 129.23, 137.10 (Ph) and 170.00 (CH_3C=O).

ES-MS(+): found *m/z* 402.11 $[M+Na]^+$ ($C_{17}H_{21}N_3O_5S$ requires M, 379.12).

Ethyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranoside 70



70

A solution of 1 M BH_3 in THF (6.45 ml, 6.45 mmol, 3 eq.) was added to compound **69** (821 mg, 2.15 mmol) at 0 ^oC. A solution of 1 M Bu_2BOTf in CH_2Cl_2 (2.15 ml, 2.15 mmol, 1 eq.) was then added slowly under stirring and the mixture was left to stir at 0 ^oC for 1 h. The reaction was then quenched with the addition of Et_3N (0.6 ml) and methanol (5 ml). The solution was then concentrated under reduced pressure followed by co-evaporation with methanol. FCC [toluene-EtOAc, (9:1)] of the residue gave the derivative **70** (672 mg, 82%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1719 (C=O), 2107 (-N_3);

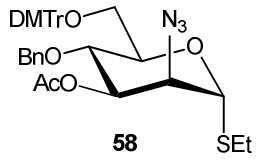
$[\alpha]_D$ +54.3 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.22 (3H, t, *J* 7.4, SCH_2CH_3), 1.99 (3H, s, Ac), 2.48-2.62 (2H, m, SCH_2CH_3), 3.72-3.76 (2H, m, H-6^{a,b}), 3.92 (1H, t, $J_{3,4} = J_{4,5} = 9.5$, H-4), 4.02 (1H, dt, $J_{5,6}$ 3.0, H-5), 4.08 (1H, dd, $J_{1,2}$ 1.5, H-2), 4.61 and 4.65 (2H, 2 x d, *J* 11.3, $PhCH_2$), 5.19 (1H, d, $J_{1,2}$ 1.5, H-1) 5.25 (1H, dd, $J_{2,3}$ 3.8, H-3) and 7.22-7.32 (5H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.85 (CH_3CH_2S), 20.75 (CH_3CO), 25.35 (CH_3CH_2S), 61.6 (C-6), 63.14 (C-2), 72.23 (C-5), 72.81 (C-4), 73.91 (C-3), 75.11 ($PhCH_2$) 82.20 (C-1), 127.81, 127.99, 128.56, 136.50 (Ph) and 172.86 (CH_3C=O).

ES-MS(+): found *m/z* 404.13 $[M+Na]^+$ ($C_{17}H_{23}N_3O_5S$ requires M, 381.14).

Ethyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-1-thio-α-D-mannopyranoside 58



58

p,p'-Dimethoxytrityl chloride (413 mg, 1.22 mmol, 1.5 eq.) was added to a solution of compound **70** (310 mg, 813 μmol) in pyridine (10 ml), after which DMAP (20 mg) was added the mixture left under stirring for 16 h. The reaction mixture was then quenched with water and diluted with CH_2Cl_2. The solution was then washed with water, dried by filtration through cotton wool and concentrated before co-evaporation with toluene. FCC [toluene-EtOAc, (19:1)→(4:1)] of the residue gave the DMTr derivative **58** (260 mg, 91%).

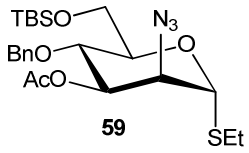
υ_{max} (film)/cm^{-1} (*inter alia*): 1722 (C=O), 2101 ($-N_3$);
$[\alpha]_D$ +37.3 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.26 (3H, t, *J* 7.4, SCH_2CH_3), 2.00 (3H, s, Ac), 2.54-2.70 (2H, m, SCH_2CH_3), 3.17 (1H, dd, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 10.2, H-6^a), 3.40 (1H, dd, $J_{5,6b}$ 1.9, H-6^b), 3.70 (6H, s, 2 x OCH_3), 3.93 (1H, t, $J_{3,4} = J_{4,5} = 9.5$, H-4), 4.07 (1H, dd, $J_{1,2}$ 1.6, H-4), 4.15 (1H, ddd, H-5), 4.19 and 4.40 (2H, 2 x d, *J* 10.9, PhCH_2) 5.20 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.33 (1H, d, $J_{1,2}$ 1.6, H-1) and 6.67-7.46 (18H, m, Ph, C_6H_4);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 14.72 (CH_3CH_2S), 20.81 (CH_3CO), 25.17 (CH_3CH_2S), 55.22 (2 x CH_3O) 62.15 (C-6), 63.02 (C-2), 71.92 (C-5), 73.46 (C-4), 73.91 (C-3), 74.84 (PhCH_2), 81.74 (C-1), 113.08, 126.72, 127.73, 127.83, 128.26, 130.13, 130.22, 135.88, 136.16, 137.67, 144.92, 158.44 (Ph, C_6H_4) and 170.00 (CH_3C=O).

ES-MS(+): found *m/z* 706.26 $[M+Na]^+$ ($C_{38}H_{41}N_3O_7S$ requires M, 683.27)

Ethyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-1-thio-α-D-mannopyranoside 59

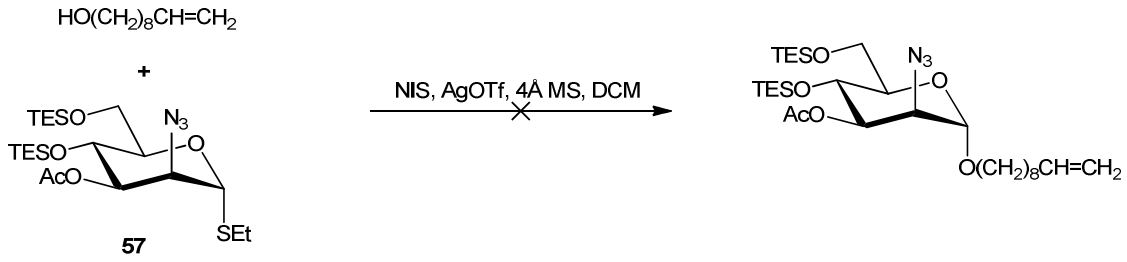


Tert-butyldimethylsilyl chloride (165 mg, 1.10 mmol, 1.5 eq.) was added to a solution of compound **70** (279 mg, 731 μmol) in pyridine (5 ml), after which DMAP (20 mg) was added and the mixture left under stirring for 16 h. The reaction mixture was then quenched with water and diluted with CH_2Cl_2. The solution was then washed with water, dried by filtration through cotton wool and concentrated before co-evaporation with toluene. FCC [toluene-EtOAc, (19:1)→(4:1)] of the residue gave the TBS derivative **59** (341 mg, 94%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1718 (C=O), 2107 ($-N_3$);
$[\alpha]_D$ +38.5 (*c* 1, $CHCl_3$);

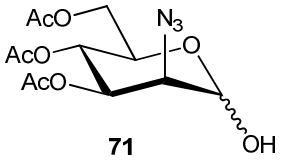
^{1}H NMR (500 MHz; $CDCl_3$): δ_H -0.02, 0.00 (6H, 2 x s, $(CH_3)_2Si$), 1.83 (9H, s, Me_3CSi), 1.20 (3H, t, *J* 7.4, SCH_2CH_3), 1.96 (3H, s, Ac), 2.46-2.62 (2H, m, SCH_2CH_3), 3.70 (1H, dd, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 11.5, H-6^a), 3.78 (1H, dd, $J_{5,6b}$ 4.5, H-6^b), 3.89 (1H, t, $J_{3,4} = J_{4,5} = 9.5$, H-4), 3.96 (1H, ddd, H-5), 4.02 (1H, dd, H-2), 4.58 and 4.61 (2H, 2 x d, *J* 11.4, $PhCH_2$), 5.19 (1H, dd, $J_{1,2}$ 1.6, H-1), 5.21 (1H, dd, $J_{2,3}$ 3.8, H-3) and 7.05-7.32 (5H, m, Ph);
^{13}C NMR (125 MHz; $CDCl_3$): δ_C -5.36, -5.15 (2 x Me_2Si) 14.68 (CH_3CH_2S), 17.77 (Me_3C) 20.81 (CH_3CO), 25.05 (CH_3CH_2S), 25.89 (Me_3C) 62.12 (C-6), 62.99 (C-2), 73.04 (C-5), 73.18 (C-4), 73.85 (C-3), 74.92 ($PhCH_2$), 81.65 (C-1), 127.72, 127.84, 128.47, 133.40 (Ph) and 170.00 (CH_3C=O).
ES-MS(+): found *m/z* 518.21 $[M+Na]^+$ ($C_{23}H_{37}N_3O_5SSi$ requires M, 495.22).

Attempted glycosylation of dec-9-en-1-ol using the thioglycosidic donor 57



A solution of the thioglycoside **57** (160 mg, 308 µmol) in CH_2Cl_2 was prepared under argon. To this dec-9-en-1-ol (71.4 µl, 400 µmol, 1.3 eq) and freshly activated 4Å molecular sieves was added. The mixture was cooled to -20 ºC and allowed to stir for 30 minutes. N-iodosuccinimide (90 mg, 400 µmol, 1.3 eq.) and silver triflate (40 mg, 156 µmol, 0.5 eq) were added and stirring continued for 4.5 hr. The reaction mixture was quenched with addition of triethylamine (250 µl) before diluting with CH_2Cl_2. The solution was filtered through celite topped with cotton wool and the filtrate was washed with 10% aq. $Na_2S_2O_3$. The organic layer was concentrated under reduced pressure. TLC analysis showed that an array of products formed.

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*α,β*-D-mannopyranose 71



71

4:1 (a:ß)

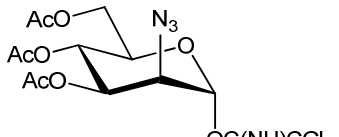
To a stirred solution of the tetra-acetate **64** (510 mg, 1.37 mmol) in THF (1 ml) was added solution of 2 M dimethylamine in THF (1.7 ml, 3.35 mmol, 2.5 eq). Stirring was continued for 0.5 h. The mixture was then concentrated under reduced pressure. FCC

[toluene-EtOAc, (9:1)] of the residue gave the hemiacetal **71** (450mg, 99%).

υ_{max} (film)/cm^{-1} (*inter alia*): 1721 (C=O), 2102 ($-N_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H (signals of the α-anomer only) 1.992, 2.042 and 2.046 (3 x s, 3 x Ac), 4.01 (dd, $J_{2,3}$ 3.7, H-2), 4.07 (dd, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.8, H-6^a), 4.10-4.18 (m, H-5, H-6^b), 5.22 (d, $J_{1,2}$ 1.8, H-1), 5.28 (t, $J_{3,4} = J_{4,5}$ 9.8, H-4) and 5.40 (dd, H-3); (selected signals of the β-anomer) 1.988, 2.038 and 2.059 (3 x s, 3 x Ac), 3.57 (1H, ddd, $J_{5,6a}$ 2.3, $J_{5,6b}$ 5.1, $J_{4,5}$ 9.9, H-5), 4.84 (1H, d, $J_{1,2}$ 1.2, H-1), 5.07 (1H, dd, $J_{2,3}$ 3.7, $J_{3,4}$ 9.9, H-3); α:β = 4:1.

3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*α*-D-mannopyranosyl trichloroacetimidate 72

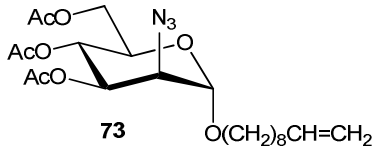


72

The hemiacetal **71** (276 mg, 833 μmol) was dissolved in dichloromethane (5 ml) under an atmosphere of argon. The solution was cooled to 0 ºC before addition of trichloroacetonitrile (251 μl, 2.50 mmol, 3 eq.) and DBU (35 μl, 250μmol, 0.3 eq.). Stirring was then continued for 1 h at 0 ºC after which the solution was concentrated under reduced pressure. FCC [toluene-EtOAc, (9:1)] of the residue gave the trichloroacetimidate derivative **72** (270 mg, 68%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 2.00, 2.03 and 2.06 (9H, 3 x s, 3 x Ac), 4.06-4.10 (2H, m, H-5, H-6^a), 4.19 (1H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 12.9, H-6^b), 4.22 (1H, dd, $J_{2,3}$ 3.3, H-2), 5.34-4.41 (2H, m, H-3, H-4), 6.23 (1H, d, $J_{1,2}$ 1.9, H-1) and 8.72 (1H, s, NH).

Dec-9-enyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside 73



A solution of the trichloroacetimidate **72** (270 mg, 568 µmol) and dec-9-en-1-ol (254 µl, 1.99 mmol, 3.5 eq) in dichloromethane (5 ml) was prepared under argon. Freshly activated 4Å molecular sieves (70 mg) were added and the mixture was cooled to 0 ºC with stirring. TMSOTf (1 µl, 5.50 µmol, 0.01 eq.) was then added and stirring continued for 0.5 h. The reaction was then quenched with triethylamine (250 µl) before diluting the mixture with chloroform. The mixture was then filtered through cotton wool and the filtrate was then washed successively three times with water. The solvent was then removed under reduced pressure. FCC [petroleum ether-EtOAc, (1:0→4:1)] of the residue gave the glycoside derivative **73** (248 mg, 93%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2103 (-N_3);

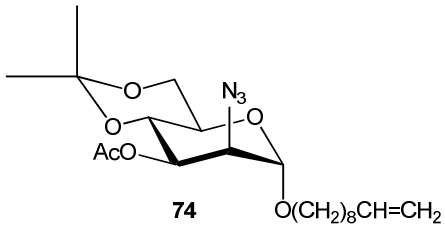
$[\alpha]_D$ +81.4 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.20-1.35 (8H, m, 4 x CH_2), 1.48-1.56 (4H, m, 2 x CH_2), 1.95-2.00 (2H, m, CH_2), 1.98, 2.03 and 2.04 (9H, 3 x s, 3 x Ac), 3.37(1H, dt, $^2J_{H,H}$ 9.6, $^3J_{H,H}$ 6.6, O*H*CHCH$_2$), 3.59 (1H, dt, OHC*H*CH$_2$), 3.85 (1H, ddd, $J_{5,6a}$ 2.3, H-5), 3.94 (1H,

dd, $J_{1,2}$ 1.7, H-2), 4.02 (1H, dd, $J_{6a,6b}$ 12.3, H-6^{a}), 4.18 (1H, dd, $J_{5,6b}$ 4.9, H-6^{b}), 4.76 (1H, d, H-1), 4.86 (1H, br d,$^{3}J_{H,H}$ 10.2, CH=HC*H*), 4.93 (1H, br d,$^{3}J_{H,H}$ 17.1, CH=*H*CH), 5.25 (1H, t, $J_{3,4} = J_{4,5} = 9.9$, H-4), 5.32 (1H, dd, $J_{2,3}$ 3.8, H-3) and 5.75 (1H, m, C*H*=CH_2); ^{13}C NMR (125 MHz; $CDCl_3$): δ_C 20.61 and 20.78 (*C*H_3CO), 26.07, 38.90, 29.07, 29.25, 29.30, 29.39, and 33.78 (C*C*H_2C), 61.69 (C-2), 62.24 (C-6), 66.05 (C-4), 68.39 (C-3), 68.51 (OCH_2), 71.17 (C-5), 97.97 (C-1), 114.21 (CH=*C*H_2), 139.17 (*C*H=CH_2) and 169.62, 169.99 and 171.13 (*C*=O).

ES-MS(+): found *m/z* 492.23 $[M+Na]^+$ ($C_{22}H_{35}N_3O_8$ requires M, 469.24).

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-4,6-*O*-isopropylidene-α-D-mannopyranoside 74



74

A solution of 4.5 M sodium methoxide in methanol (250 µl) was added to a stirring solution of compound **73** (245 mg, 522 µmol) in a mixture of CH_2Cl_2 (1 ml) and methanol (6 ml). The solution was then stirred at room temperature for 1 h prior the addition of DOWEX 50WX4-50 (H^+) resin. The resin was then filtered off and the filtrate was concentrated under reduced pressure. The residue was co-evaporated with toluene (3 x 20 ml) and then dissolved in a mixture of DMF (2 ml) and dry acetone (400 µl). 2,2-Dimethoxypropane (478 µl, 3.90 mmol) and toluenesulphonic acid (10 mg) were then added and the solution was stirred at room temperature for 16 h. Pyridine (2

ml) and acetic anhydride (600 µl) were then added and stirring was continued for 2.5 h. Water was then added and the mixture was extracted with EtOAc. The organic layer was washed successively with 1 M HCl, water, saturated aq. $NaHCO_3$ and dried (by filtration though cotton wool) before concentrating under reduced pressure. FCC [toluene-EtOAc, (19:1)] of the residue gave the derivative **74** (172 mg, 78%).

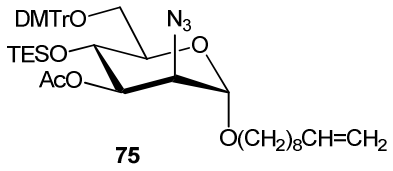
υ_{max} (film)/cm^{-1} (*inter alia*): 2101 ($-N_3$);

$[\alpha]_D$ +72.8 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.20-1.35 (8H, m, 4 x CH_2), 1.33 (3H, s, CC*H*$_3$), 1.45 (3H, s, CC*H*$_3$), 1.46-1.55 (4H, m, 2 x CH_2), 1.95-2.00 (2H, m, CH_2), 2.07 (3H, s, Ac), 3.30 (1H, dt, $^2J_{H,H}$ 9.6, $^3J_{H,H}$ 6.6, O*H*CHCH$_2$), 3.57 (1H, dt, OHC*H*CH$_2$), 3.64 (1H, m, H-5), 3.70-3.78 (2H, m, H-6a,b), 3.98 (1H, t, $J_{3,4} = J_{4,5}$ = 9.8, H-4), 4.03 (1H, dd, $J_{1,2}$ 1.4, H-2), 4.66 (1H, d, H-1), 4.86 (1H, br d,$^3J_{H,H}$ 10.2, CH=HC*H*), 4.93 (1H, br d,$^3J_{H,H}$ 17.1, CH=*H*CH), 5.20 (1H, dd, $J_{2,3}$ 3.9, H-3) and 5.75 (1H, m, C*H*=CH$_2$);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C (selected signals) 19.22 (*C*H$_3$C), 20.75 (*C*H$_3$CO), 26.04, 28.90, 29.13, 29.28, 33.78 (C*C*H$_2$C and *C*H$_3$C), 62.24 (C-2), 64.67 (C-6), 68.25, 68.88, 70.73 (C-3, C-4, C-5 and OCH$_2$), 98.87 (C-1) and 114.21 (C=*C*H$_2$).

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-*α*-D-mannopyranoside 75



75

Trifluoroacetic acid (1 ml) and water (33 μl) were added to a stirring solution of compound **74** (165 mg, 391 μmol) in CH_2Cl_2 (10 ml). The solution was stirred at room temperature for 30 min before concentrating under reduced pressure, followed by co-evaporation with toluene. The residue was then dissolved in pyridine (2 ml) followed by the addition of *p,p'*-dimethoxytrityl chloride (172 mg, 508 μmol, 1.3 eq.) and the solution was stirred for 16 h. Triethylamine (500 μl) was then added and the solution cooled to 0 ºC followed by the addition TESOTf (114 μl, 508 μmol, 1.3 eq). The solution was stirred at 0 °C for 3 h, water (1 ml) was added and stirring was continued for a further 1 h. The solution was then diluted with dichloromethane, washed with saturated aq. $NaHCO_3$, dried by filtration through cotton wool before concentrating under reduced pressure. FCC [petroleum ether-EtOAc, (1:0→4:1)] of the residue gave the derivative **75** (245 mg, 78%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

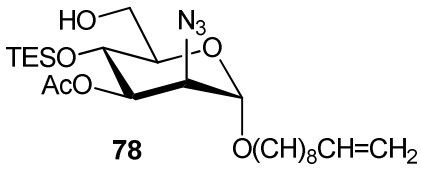
$[\alpha]_D$ +48.3 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 0.25-0.38 (6H, m, 3 x CH_3CH_2Si), 0.75 (9H, t, *J* 8.0, 3 x CH_3CH_2Si), 1.25 (12H, m, 6 x CH_2), 1.67 (2H, m, CH_2), 2.02 (2H, m, CH_2), 2.12 (3H, s, Ac), 3.17 (1H, dd, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 9.5, H-6^a), 3.30 (1H, dd, $J_{5,6b}$ 1.4, H-6^b), 3.52(1H, dt,

$^{2}J_{H,H}$ 9.6, $^{3}J_{H,H}$ 6.6, O*H*CHCH$_2$), 3.78 (6H, 2 x s, 2 x OMe), 3.82 (1H, t, $J_{3,4} = J_{4,5} = 9.4$, H-4), 3.85-3.93 (2H, m, O*H*CHCH$_2$ and H-2), 4.05 (1H, dd, $J_{1,2}$ 1.6, H-2), 4.85 (1H, d, H-1), 4.92 (1H, br d,$^{3}J_{H,H}$ 10.2, CH=HC*H*), 4.98 (1H, br d,$^{3}J_{H,H}$ 17.1, CH=*H*CH), 5.11 (1H, dd, $J_{2,3}$ 3.9, H-3), 5.80 (1H, m, C*H*=CH$_2$) and 6.78-7.49 (13H, m, Ph, C$_6$H$_4$).

ES-MS(+): found *m/z* 824.43 $[M+Na]^+$ ($C_{45}H_{63}N_3O_8S$ requires M, 801.44).

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranoside 78



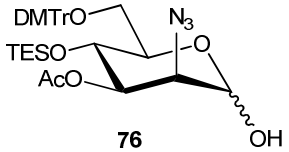
78

The dec-9-enyl glycoside **75** (100 mg, 125 μmol) was dissolved in CH_2Cl_2 (5 ml) and the solution was cooled to 0 ºC under stirring. A mixture of CH_2Cl_2-TFA (49:1, 5 ml) was then prepared and cooled to 0 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition ofsaturated aq. $NaHCO_3$. The solution was then diluted with CH_2Cl_2, washed with saturated aq. $NaHCO_3$, water, dried by filtration though through cotton wool and concentrated under reduce pressure. FCC [petroleum ether-EtOAc, (1:0→4:1)] of the residue gave the derivative **78** (60 mg, 96%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 0.25-0.38 (6H, m, 3 x CH$_3$C*H*$_2$Si), 0.75 (9H, t, *J* 8.0, 3 x C*H*$_3$CH$_2$Si), 1.25 (12H, m, 6 x CH$_2$), 1.67 (2H, m, CH$_2$), 2.02 (2H, m, CH$_2$), 2.12 (3H, s, Ac), 3.17 (1H, dd, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 9.5, H-6^{a}), 3.30 (1H, dd, $J_{5,6b}$ 1.4, H-6^{b}), 3.52(1H, dt, $^{2}J_{H,H}$ 9.6, $^{3}J_{H,H}$ 6.6, O*H*CHCH$_2$), 3.78 (6H, 2 x s, 2 x OMe), 3.82 (1H, t, $J_{3,4} = J_{4,5} = 9.4$, H-

4), 3.85-3.93 (2H, m, O*H*CHCH$_2$ and H-2), 4.05 (1H, dd, $J_{1,2}$ 1.6, H-2), 4.85 (1H, d, H-1), 4.92 (1H, br d,$^3J_{H,H}$ 10.2, CH=HC*H*), 4.98 (1H, br d,$^3J_{H,H}$ 17.1, CH=*H*CH), 5.11 (1H, dd, $J_{2,3}$ 3.9, H-3) and 5.80 (1H, m, C*H*=CH$_2$).

3-*O*-Acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-α,β-D-mannopyranose 76



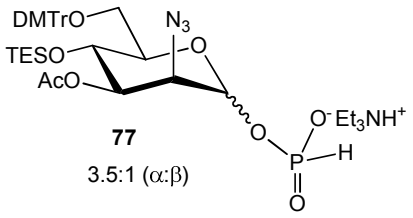
76
3.5:1 (α:β)

The thioglycoside **56** (270 mg, 381 μmol) was dissolved in wet dichloromethane (10 ml) and cooled to -20 ºC with stirring. N-iodosuccinimide (103 mg, 457 μmol, 1.2 eq) and silver triflate (5 mg, 19 μmol, 0.05 eq) was then added and stirring was continued at -20 ºC for 0.5 h. Two drops of water (~66 μl) was then added and the mixture was stirred for another 0.5 h. The reaction was then diluted with dichloromethane and the solution was washed successively with 0.5 aq. $Na_2S_2O_2$, water and dried by filtration through cotton wool. The solvent was then removed under reduced pressure. FCC [toluene-EtOAc, (19:1)] of the residue gave the hemiacetal derivative **76** (100 mg, 40%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H(signals of the α-anomer only) 0.26-0.39 (m, 3 x CH_3CH_2Si), 0.75 (m,3 x CH_3CH_2Si) 2.14 (s, Ac), 2.89 (d, $J_{1,OH}$ 3.4, 1-OH) 3.17 (dd, $J_{6a,6b}$ 9.9, H-6^a), 3.38 (dd, $J_{5,6b}$ 1.9, H-6^b), 3.78 (s, 2 x OMe), 3.96 (t, $J_{3,4} = J_{4,5} = 9.2$, H-4), 4.08 (ddd, $J_{5,6a}$ 6.7, H-5), 4.12 (dd, $J_{1,2}$ 1.9,H-2), 5.19 (dd, $J_{2,3}$ 3.6, H-3), 5.21 (m, H-1) and

6.80-7.51 (m, Ph, 2 x C_6H_4); (selected signals of the β-anomer) 2.16 (s, Ac), 3.38 (d, $J_{1,OH}$ 10.0, 1-OH),3.48 (m,H-5),3.92 (t, $J_{3,4} = J_{4,5} = 9.1$, H-4), 4.15 (dd, $J_{2,3}$ 3.6,H-2), 4.84 (dd,H-3), 4.95 (dd, $J_{1,2}$ 1.3, H-1); α:β = 3.4:1.

Triethylammonium 3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-*α,β*-D-mannopyranosyl hydrogenphosphonate 77



77
3.5:1 (α:β)

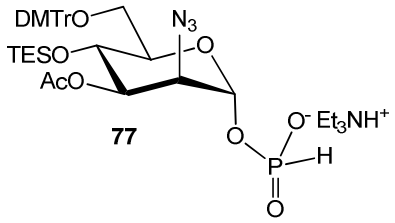
To a stirred solution of imidazole (130 mg, 1.90 mmol, 14 eq) in MeCN (3.5 ml) at 0 ºC was added PCl_3 (47 µl, 544 µmol, 4 eq) and then triethylamine (284 µl, 2.04 mmol, 15 eq). The mixture was stirred for 0.5 h. A solution of the hemiacetal **76** (90 mg, 136 µmol) in MeCN (3.5 ml) was then added dropwise at 0 ºC over 0.5 h. Cooling was then ceased and the mixture was allowed to warm to room temperature with stirring for 0.5 h before 0.5 M TEAB (triethylammonium hydrogen carbonate) buffer (pH 7) was added to quench the reaction. To clear solution chloroform was added, and the organic layer was then washed in turn twice with water, twice with 0.5 M TEAB buffer and dried by filtration through cotton wool. The solvent was then removed under reduced pressure. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the H-phosphonate **77** (95 mg, 84%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H(signals of the α-anomer only) 0.15-0.30 (m, Si(CH_2CH_3)$_3$), 0.66 (t, *J* 8.0, Si(CH_2CH_3)$_3$) 1.27 (t, *J* 7.3, 3 x NCH_2CH_3), 2.05 (s, Ac),

3.00 (q, 3 x NCH_2CH_3), 3.08 (dd, $J_{5,6a}$ 3.3, $J_{6a,\ 6b}$ 9.4, H-6^a), 3.26 (dd, $J_{5,6b}$ 1.7, H-6^b), 3.71 (s, 2 x OMe), 3.92 (t, $J_{3,4} = J_{4,5}$ = 9.4, H-4), 4.01-4.04 (m, H-5), 4.05 (dd, $J_{1,2}$ 2.0, H-2), 5.13 (dd, $J_{2,3}$ 3.8, H-3), 5.67 (dd, , $J_{1,P}$ 8.7, H-1), 7.09 (d, $J_{P,H}$ 635.6, PH) and 6.69-7.47 (m, Ph, C_6H_4); (selected signals of the β-anomer) 4.08 (dd, $J_{2,3}$ 3.7,H-2), 4.69 (dd, $J_{3,4}$ 9.3,H-3), 4.45 (dd, $J_{1,2}$ 1.0, $J_{1,P}$ 8.8, H-1), 7.15 (1H, d, $J_{P,H}$ 641.8, PH);

^{31}P NMR (202 MHz; $CDCl_3$): δ_P 0.22 (α-anomer) and 0.59(β-anomer); α:β = 3.2:1 .

Triethylammonium 3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-*α*-D-mannopyranosyl hydrogenphosphonate 77



The H-phosphonate **77** (85 mg, 102 μmol) was dissolved in a mixture of CH_2Cl_2 (2.1 ml) and methanol (0.5 ml). A solution of silver triflate (50 mg, 200 μmol, 2 eq.) in toluene (1 ml) was added under stirring. Stirring was then continued for 45 min before quenching the reaction with the addition of 0.5 M TEAB buffer, followed by the aqueous work-up as described for the preparation of compound **77**. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the pure α-H-phosphonate derivative **77** (46 mg, 37%).

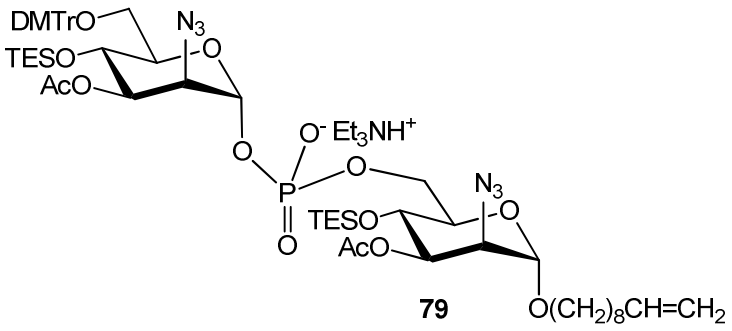
$[\alpha]_D$ +29.4 (*c* 1, $CHCl_3$);

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

δ_H 0.15-0.30 (6H, m, Si(CH_2CH$_3$)$_3$), 0.66 (9H, t, *J* 8.0, Si(CH$_2$$CH_3$)$_3$) 1.27 (9H, t, 3 x C$H_3CH_2$N), 2.05 (3H, s, Ac), 3.00 (6H, q, 3 x CH_3CH$_2$N), 3.08 (1H, dd, $J_{5,6a}$ 3.3, $J_{6a,\ 6b}$ 9.4, H-6^a), 3.26 (1H, dd, $J_{5,6b}$ 1.7, H-6^b), 3.71 (6H, s, 2 x OMe), 3.92 (1H, t, $J_{3,4} = J_{4,5} =$ 9.4, H-4), 4.01-4.04 (1H, m, H-5), 4.05 (1H, dd, $J_{1,2}$ 2.0, H-2), 5.13 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.67 (1H, dd, $J_{1,P}$ 8.7, H-1), 7.12 (1H, d, $J_{P,H}$ 635.4, PH) and 6.69-7.47 (13H, m, Ph, C_6H_4);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P 0.17.

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranoside 6-[3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-α-D-mannopyranosyl phosphate], triethylammonium salt 79



79

A mixture of compounds **77** (46 mg, 56 µmol, 1.2 eq.) and **78** (23 mg, 46 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, prior trimethylacetyl chloride (17 µl, 138 µmol, 2.4 eq) was added, and the mixture was stirred for at 20 ^{o}C for 40 min. The mixture was cooled to -40 ºC before a freshly prepared solution of iodine (24 mg, 92 µmol, 2 eq.) in pyridine-water (95:5; 1 ml) was added. The mixture was then allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture,

and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$, cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the phosphodiester derivative**79** (49 mg, 75%).

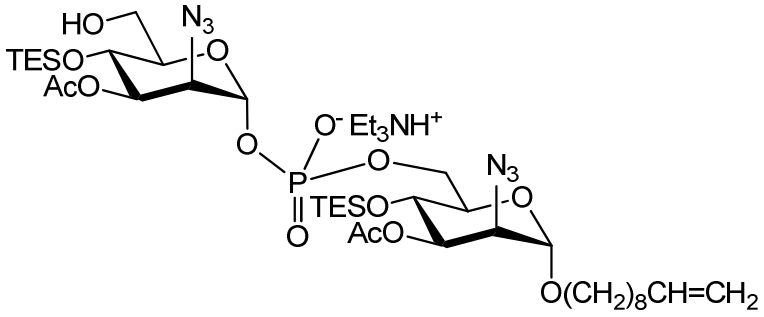
υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +19.4 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H0.26 (6H, m, 3 x Si$CH_2$$CH_3$), 0.63 (6H, q, *J* 7.8, 3 x Si$CH_2$$CH_3$), 0.71 (9H, t, *J* 7.8, 3 x Si$CH_2$$CH_3$), 0.93 (9H, t, *J* 7.9, 3 x Si$CH_2$$CH_3$) 1.10-1.42 (12H, m, 6 x CH_2), 1.29 (9H, t, *J* 7.5, 3 x $CH_3$$CH_2$N) 1.97-2.03 (2H, m, CH_2), 2.10 and 2.13 (6H, 2 x s, 2 x Ac), 3.03 (6H, m, 3 x $CH_3$$CH_2$N), 3.10-3.18 (2H, m, OHCHCH_2 and H-6$^{a'}$), 3.33 (1H, m, H-6$^{b'}$) 3.45-3.52 (1H, m, OHCHCH_2), 3.68 (1H, m, H-5), 3.75 (6H, s, 2 x OMe), 4.00 (1H, dd, $J_{1,2}$ 1.5, H-2), 4.03 (1H, t, $J_{3,4} = J_{4,5}$ = 9.3, H-4), 4.04-4.09 (2H, m, H-5'and H-6^a), 4.10 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.3, H-4'), 4.20 (1H, m, H-6^b), 4.30 (1H, dd, $J_{1',2'}$ 2.0, H-2'), 4.63 (1H, d, H-1), 4.90 (1H, br d,$^3J_{H,H}$ 10.2, CH=HC*H*), 4.96 (1H, br d,$^3J_{H,H}$ 17.1, CH=*H*CH), 5.11 (1H, dd, $J_{2,3}$3.8, H-3), 5.26 (1H, dd, $J_{2',3'}$ 3.6, H-3'), 5.67 (1H, dd, $J_{1,P}$ 7.9, H-1'), 5.78 (1H, m, C*H*=CH_2) and 6.74-7.49 (13H, m, Ph, 2 x C_6H_4);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.13.

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranoside 6-(3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranosyl phosphate), triethylammonium salt 80



80

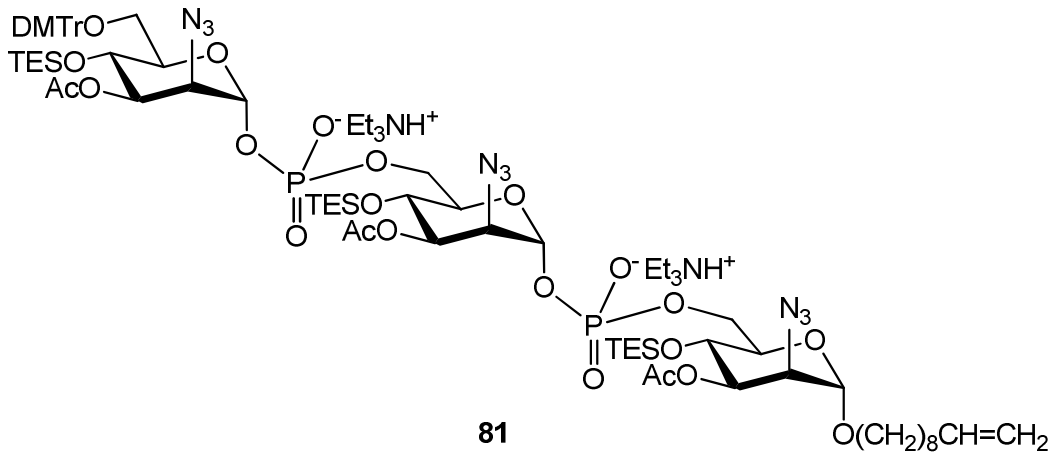
The disaccharide phosphate **79** (52 mg, 65 μmol) was dissolved in CH_2Cl_2 (5 ml) and cooled to 0 ºC with stirring. A mixture of CH_2Cl_2-TFA (49:1, 5 ml) was then prepared and cooled to 0 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition ofsaturated aq. $NaHCO_3$. The mixture was then diluted with CH_2Cl_2, washed successively with saturated aq. $NaHCO_3$, 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated under reduce pressure. FCC [CH_2Cl_2-methanol-Et_3N, (90:10:1)] of the residue gave the phosphodiester derivative **80** (31 mg, 96%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H0.52-0.61 (12H, m, 6 x SiC*H*$_2$CH$_3$), 0.86-0.91 (18H, m, 6 x SiCH$_2$C*H*$_3$), 1.17-1.33 (10H, m, 5 x CH_2), 1.25 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$N), 1.45-1.51 (2H, m, CH_2), 1.94-2.00 (2H, m, CH_2), 2.07 and 2.09 (6H, 2 x s, 2 x Ac), 2.99 (6H, q, 3 x CH$_3$C*H*$_2$N), 3.24-3.30 (1H, m, O*H*CHCH$_2$), 3.56-3.68 (3H, m, H-5, H-6$^{a'}$ and OHC*H*CH$_2$), 3.75 (1H, dd, $J_{5',6b'}$ 1.7, $J_{6a',6b'}$ 10.5, H-6$^{b'}$),3.89-4.00 (5H, m, H-2, H-4, H-4', H-5' and H-6^a), 4.09-4.13 (2H, m, H-2' and H-6^b), 4.63 (1H, d, $J_{1,2}$ 1.5, H-1), 4.86 (1H, br d,$^3J_{H,H}$ 10.2, CH=HC*H*), 4.92 (1H, br d,$^3J_{H,H}$ 17.1, CH=*H*CH), 5.07 (1H, dd,

$J_{2,3}$3.8, $J_{3,4}$9.3, H-3), 5.17 (1H, dd, $J_{2',3'}$ 3.9, $J_{3',4'}$ 9.8, H-3'), 5.48 (1H, dd, $J_{1,2}$ 1.7, $J_{1,P}$ 7.1, H-1') and 5.75 (1H, m, C*H*=CH_2);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -2.58.

Dec-9-enyl 3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranoside 6-{3-*O*-acetyl-2-azido-2-deoxy-4-*O*-triethylsilyl-α-D-mannopyranosyl phosphate 6-[3-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-4-*O*-triethylsilyl-α-D-mannopyranosyl phosphate]}, bis-triethylammonium salt 81



81

A mixture of the H-phosphonate **77** (33mg, 36 µmol, 1.2 eq) and the disaccharide phosphate **80** (31 mg, 30 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, prior trimethylacetyl chloride (12 µl, 91 µmol, 3 eq.) was added, and the mixture was stirred for at 20 ^{o}C for 40 min. The mixture was cooled to -40 ºC before a freshly prepared solution of iodine (15 mg, 61 µmol, 2 eq) in pyridine-water (95:5; 1 ml) was added. The mixture was then allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$, cold 0.5 M TEAB buffer, dried by filtration through cotton

wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→85:15:1)] of the residue gave the phosphosaccharide derivative **81** (28 mg, 50%).

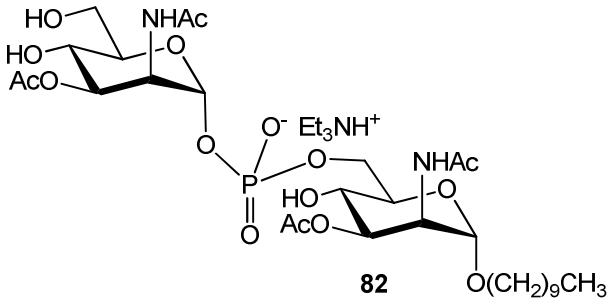
υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +10.7 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H0.21 (6H, m, 3 x Si$CH_2$$CH_3$), 0.55 (12H, m, 6 x Si$CH_2$$CH_3$), 0.66 (9H, t, *J* 7.8, 3 x $SiCH_2CH_3$), 0.79-0.91 (18H, m, 6 x $SiCH_2CH_3$),1.21 (18H, t, 6 x CH_3CH_2N), 1.27-1.33 (2H, m, CH_2),1.45-1.55 (8H, m, 4 x CH_2),1.93-1.98 (4H, m, 2 x CH_2), 2.06 and 2.08 (9H, 2 x s, 3 x Ac), 2.97 (12H, q, 6 x CH_3CH_2N), 3.06 (1H, dd, $J_{5'',6a''}$ 4.4, $J_{6a'',6b''}$ 9.8, H-6^{a}''), 3.24 (1H, m, O*H*CHCH_2), 3.29-3.33(1H, m, H-6^{b}''), 3.59-3.67 (2H, m, OHC*H*CH_2 and H-5), 3.71 (6H, s, 2 x OMe), 3.89-4.02 (8H, m, H-2, H-4, H-4', H-4'', H-5', H-5'', H-6^{a} and H-6^{a}'), 4.05-4.26 (4H, m, H-2', H-2'', H-6b and H-6b'), 4.62 (1H, br s, H-1), 4.85 (1H, br d,$^3J_{H,H}$ 10.2, CH=HC*H*), 4.92 (1H, br d,$^3J_{H,H}$ 17.1, CH=*H*CH), 5.07 (1H, dd, $J_{2,3}$3.8, $J_{3,4}$9.3, H-3), 5.18 (1H, dd, $J_{2'',3''}$ 3.8, $J_{3'',4''}$9.3, H-3''), 5.25 (1H, dd, $J_{2',3'}$ 3.5, $J_{3',4'}$9.6, H-3'), 5.41 (1H, dd, $J_{1',2'}$1.1, $J_{1',P}$ 7.9, H-1'), 5.60 (1H, dd, $J_{1'',2''}$1.6, $J_{1'',P}$ 7.7, H-1''), 5.74 (1H, m, C*H*=CH_2) and 6.70-7.45 (13H, m, Ph, C_6H_4);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.42.

Dec-9-enyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranoside 6-(2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate), triethylammonium salt 82



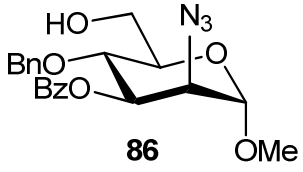
82

The disaccharide phosphate **79** (18 mg, 13μmol) was dissolved in a mixture of THF (0.5 ml) and MeOH (0.5 ml) before the addition of $NiCl_2 \cdot 6H_2O$ (25 mg). The mixture was then cooled to 0 ºC with stirring. In portions, $NaBH_4$ (30 mg) was added slowly over the course of 1 h, after which time AcO_2 (100 μl) was added. The solution was stirred for an additional 1 h. The reaction was then diluted with CH_2Cl_2 and washed with 0.5 M TEAB buffer. The organic layer was filtered through silica and concentrated under reduced pressure. The residue was then dissolved in CH_2Cl_2 (3 ml) and cooled to 0 $^{\circ}$C. A further solution of TFA (60 μl) in CH_2Cl_2 (3 ml) and was prepared and cooled to 0 ºC. The solutions were combined and stirred for 1.5 min, and the reaction was quenched with saturated aq. $NaHCO_3$. The mixture was then diluted with CH_2Cl_2, washed successively with saturated aq. $NaHCO_3$, 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated under reduce pressure. The residue was dissolved in THF (0.5 ml) before the addition of Et_3N (15 μl) and $Et_3N \cdot 3HF$ (15 μl, 92 μmol). The reaction was then stirred at ambient temperature for 2 h then quenched with a solution of Et_3SiOMe before concentrating under reduced pressure. The residue was then triturated with ether to remove any silylated products to give the phosphodiester **82** (4 mg, 40 %) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ_H0.81 (3H, t, *J* 6.9, CH_3, *n*-decyl), 1.16-1.32 (25H, m, 8 x CH_2, *n*-decyl, 3 x C*H*$_3$CH_2N), 1.95 (12H, s, 2 x OAc, 2 x NAc), 3.10 (6H, q, *J* 7.5, 3 x CH_3C*H*$_2$N), 3.26 (1H, m, O*H*CHCH_2, *n*-decyl), 3.47-3.63 (3H, m, H-4, H-4' and OHC*H*CH_2, *n*-decyl), 3.79-4.02 (5H, m, H-5, H-5', H-6^a, H-6$^{a'}$, H-6$^{b'}$),4.33 (1H, m, H-6^b), 4.53 (2H, m, H-2 and H-2'), 4.56 (1H, br, H-1), 5.07 and 5.16 (2H, 2 x d, $J_{2,3}$ 4.3, $J_{3,4}$ 10.0, $J_{2',3'}$ 4.3, $J_{3',4'}$ 10.0, H-3 and H-3'), 5.47 (1H, br d, $J_{1',P}$ 7.3, H-1');

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -1.30.

Methyl 2-azido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 86



A solution of 1 M BH_3 in THF (3 ml, 3 mmol) was added to compound **62** (567 mg, 1.38 mmol) at 0 °C. A solution of 1 M Bu_2BOTf in CH_2Cl_2 (1.4 ml, 1.4 mmol) was then added slowly under stirring and the mixture was left to stir at 0 °C for 1 h. The reaction was then quenched with the addition of Et_3N (0.6 ml) before dropwise addition of methanol (5 ml). The solution was then concentrated under reduced pressure followed by co-evaporation with methanol (3 x 30 ml). FCC [toluene-EtOAc, (9:1)] of the residue gave the derivative **86** (533 mg, 94%).

υ_{max} (film)/cm-1 (*inter alia*): 1723 (C=O), 2103 (-N_3);

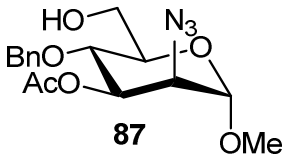
$[\alpha]_D$ +53.1 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.85 (1H, m, 6-OH), 3.33 (3H, s,OMe), 3.69 (1H, dt, $J_{5,6}$ 3.1, H-5), 3.71-3.82 (2H, m, H-6^{a,b}), 4.06 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, H-4), 4.09 (1H, dd, H-2), 4.58 and 4.65 (2H, 2 x d, *J* 11.0, Ph*CH*$_2$), 4.68 (1H, d, $J_{1,2}$ 1.5, H-1) 5.63 (1H, dd, $J_{2,3}$ 3.8, H-3) and 7.05-8.07 (10H, m, 2 x Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 55.16 (CH_3O), 61.69 (C-6), 62.09 (C-2), 71.89 (C-5), 72.64 (C-4), 73.85 (C-3), 75.18 (Ph*C*H$_2$), 99.16 (C-1), 127.96, 128.05, 128.45, 128.63, 129.23, 129.90, 133.54 (Ph) and 165.49 (*C*=O).

ES-MS(+): found *m/z* 436.15 $[M+Na]^+$ ($C_{21}H_{23}N_3O_6$ requires M, 413.16).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 87



A solution of compound **86** (491 mg, 1.19 mmol) in CH_2Cl_2 (10 ml) and methanol (5 ml) was prepared with stirring. To this sodium methoxide (solid, ~10 mg) was added. The solution was then stirred at room temperature for 2 h and then deionised with the addition of DOWEX 50WX4-50 (H^+) resin. The resin was filtered off before filtrate was concentrated. The residue was co-evaporated with toluene (3 x 30 ml) and dissolved in pyridine (10 ml) with stirring prior to the addition of *p,p'*-dimethoxytrityl chloride (484 mg, 1.43 mmol, 1.2 eq). The solution was then stirred at ambient temperature for 16 h, after which time acetic anhydride (2 ml) was added. The solution was stirred for an additional 1 h before quenching with the addition of water. The mixture was diluted with CH_2Cl_2, washed successively with saturated aq. $NaHCO_3$, water and dried by

filtration through cotton wool and concentrated. The residuewas dissolved in CH_2Cl_2 (10 ml) and cooled to 0 ºC with stirring. A mixture of CH_2Cl_2-TFA (49:1, 10 ml) was then prepared and cooled to 0 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition ofsaturated aq. $NaHCO_3$. The solution was then diluted with CH_2Cl_2, washed with saturated aq. $NaHCO_3$, dried by filtration through cotton wool and concentrated. FCC [toluene-EtOAc, (9:1)] of the residue gave the derivative **87** (326 mg, 78%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2106 ($-N_3$);

$[\alpha]_D$ +34.5 (*c* 1, $CHCl_3$);

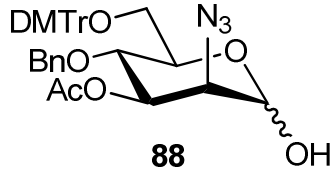
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.99 (3H, s, Ac), 3.30 (3H, s,OCH_3), 3.62 (1H, ddd, $J_{5,6a}$ 3.8, H-5), 3.71 (1H, dd, $J_{5,6a}$ 3.8, $J_{6a,\ 6b}$ 12.0, H-6^a), 3.77 (1H, dd, $J_{5,6b}$ 2.6, H-6^b), 3.89 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, H-4), 3.98 (1H, dd, H-2), 4.60 and 4.65 (2H, 2 x d, *J* 11.3, Ph*CH*$_2$), 4.61 (1H, d, $J_{1,2}$ 1.7, H-1) 5.31 (1H, dd, $J_{2,3}$ 3.8, H-3) and 7.21-7.31 (5H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 20.76 (*C*H_3CO), 55.10 (CH_3O), 61.66 (C-6), 61.75 (C-2), 71.79 (C-5), 72.61 (C-4), 73.62 (C-3), 75.06 (Ph*C*H_2), 99.04 (C-1), 127.76, 127.93, 128.51 137.89 (Ph) and 170.02 (*C*=O).

ES-MS(+): found *m/z* 374.13 $[M+Na]^+$ ($C_{16}H_{21}N_3O_6$ requires M, 351.14).

High resolution ES-MS(+): found *m/z* 374.1342 $[M+Na]^+$ ($C_{16}H_{21}N_3NaO_6^+$ requires *m/z* 374.1323).

3-*O*-Acetyl-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-*α,β*-D-mannopyranose 88

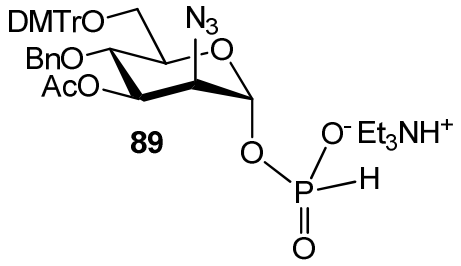


88
4.5:1 (α:β)

The thioglycoside **58** (1.37 g, 2.00 mmol) was dissolved in dichloromethane (10 ml) and cooled to 0 ºC with stirring. Bromine (109 µl, 2.00 mmol, 1 eq.) was then added, stirring was continued at 0 ºC for 30 min. The reaction mixture was then diluted with acetone-water (9:1; 50 ml) before the addition of silver carbonate (400 mg). Cooling was ceased and the mixture was stirred for an additional 16 h at room temperature. The suspension was then diluted with toluene, filtered through a Celite pad, and the filtrate was dried by filtration through cotton wool and concentrated. FCC [toluene-EtOAc, (9:1)] of the residue gave the hemiacetal derivative **88** (1.13 g, 89%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H(signals of the α-anomer only) 2.00 (s, Ac), 2.71 (d, $J_{1,OH}$ 3.4, 1-OH) 3.15 (m, H-6^a), 3.42 (dd, $J_{5,6b}$ 1.8, $J_{6a,6b}$ 10.3 H-6^b), 3.70 (s, 2 x OMe), 3.98 (ddd, $J_{5,6a}$ 3.6, $J_{4,5}$ = 9.0, H-5), 4.02 (m, H-4), 4.04 (m, H-2), 4.20 and 4.42 (2 x d, J 11.0, PhCH_2), 5.26 (dd, $J_{1,2}$ 1.9, H-1), 5.33 (dd, $J_{2,3}$ 3.8, $J_{3,4}$ 9.0, H-3) and 6.72-7.46 (m, Ph, 2 x C_6H_4); (signals of the β-anomer) 2.02 (s, Ac), 3.15 (m, H-6^a), 3.35 (d, $J_{1,OH}$ 10.2, 1-OH), 3.38 (ddd, $J_{5,6a}$ 3.5, $J_{4,5}$ 9.7, H-5), 3.50 (dd, $J_{5,6b}$ 1.9, $J_{6a,6b}$ 10.3, H-6^b), 3.70 (s, 2 x OMe), 4.02 (m, H-4), 4.07 (dd, $J_{2,3}$ 3.7,H-2), 4.21 and 4.39 (2 x d, J 10.9, PhCH_2), 4.83 (dd, $J_{1,2}$ 1.5, H-1), 4.97 (dd, $J_{3,4}$ 9.8, H-3) and 6.72-7.46 (m, Ph, 2 x C_6H_4);α:β = 4.5:1.

Triethylammonium 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-α-D-mannopyranosyl hydrogenphosphonate 89



The hemiacetal **88** (232 mg, 363 μmol) was dissolved in a mixture of dioxane (10 ml) and Et_3N (7 ml) and with stirring for 1 h. Salicylchlorophosphite (88 mg, 436 μmol, 1.2 eq) was then added and the mixture was stirred for 1 h. The reaction was then quenched with the addition of saturated aq. $NaHCO_3$ and stirring continued for additional 30 min. The mixture was then diluted with chloroform (80 ml) and the solution was washed successively with saturated aq. $NaHCO_3$, water, cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the H-phosphonate derivative **89** (257 mg, 88%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2103 (-N_3);

$[\alpha]_D$ +32.8 (*c* 1, $CHCl_3$);

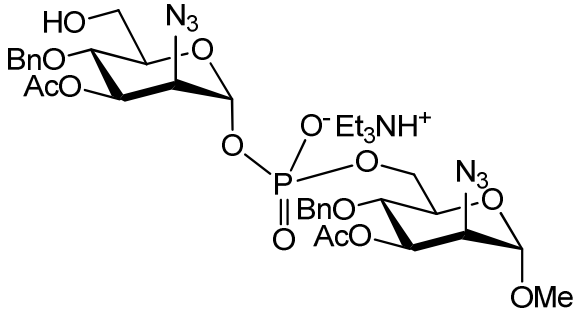
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.24 (9H, t, 3 x C*H*$_3$ CH_2NH^+), 1.97 (3H, s, CO*CH*$_3$), 2.97 (6H, q, 3 x CH_3 C*H*$_2$$NH^+$), 3.12 (1H, dd, $J_{5,6a}$ 3.6, $J_{6a,\ 6b}$ 10.3, H-6^a), 3.43 (1H, dd, $J_{5,6b}$ 1.7, H-6^b), 3.69 (6H, s, 2 x O-Me), 3.99 (1H, ddd, H-5), 4.06 (1H, dd, $J_{1,2}$ 2.0, H-2), 4.10 (1H, t, $J_{3,4}$ =$J_{4,5}$ 9.8, H-4), 4.20 and 4.40 (2H, 2 x d, $PhCH_2$), 5.32 (1H, dd, $J_{2,3}$ 3.7, H-3), 5.69 (1H, dd, , $J_{1,P}$ 8.6, H-1), 6.96 (1H, d, $J_{P,H}$ 633.2, PH) and 6.71-7.47 (18H, m, Ph, C_6H_4);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 10.19 (CH_3CH_2N), 20.85 (CH_3CO), 45.88 (CH_3CH_2N) 55.20 (2 x CH_3O) 61.88 (C-6), 62.62 (d, $^3J_{C,P}$ 6.5, C-2), 72.54, 72.87 and 73.46 (C-3, C-4 and C-5), 74.72 ($PhCH_2$), 85.63 (Ar_3C), 93.36 (d, $^2J_{C,P}$ 3.8, C-1), 113.07-158.35 (Ph, C_6H_4) and 170.18 (CH_3C=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P 0.22.

High resolution ES-MS(-): found *m/z* 702.2251 $[M\text{-}Et_3N\text{-}H]^-$ ($C_{36}H_{37}N_3O_{10}P^-$ requires *m/z* 702.2222).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate), triethylammonium salt 92



92

A mixture of the H-phosphonate **89** (300 mg, 376 µmol, 1.2 eq) and compound **87** (110 mg, 313 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (70 µl, 564 µmol, 1.8 eq.) was added, and the solution was stirred for at 20 ^oC for 30 min. The solution was cooled to -40 ºC before Et_3N (210 µl, 1.50 mmol, 4.8 eq.) and a

mixture of pyridine-water (95:5; 1 ml) were added. Iodine (191 mg, 752 μmol, 2.4 eq) was then added, the mixture was stirred for 30 min at -40 ºC, prior it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$, cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. The residuewas dissolved in CH_2Cl_2 (5 ml) and cooled to -10 ºC with stirring. A mixture of DCM-TFA (49:1, 5 ml) was then prepared and cooled to -10 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition ofsaturated aq. $NaHCO_3$. The solution was then diluted with chloroform and washed successively with saturated aq. $NaHCO_3$ and cold 0.5 M TEAB buffer, by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the phosphodiester **92** (235 mg, 88%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +23.3 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.20 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$NH$^+$), 1.94 and 1.96 (6H, 2 x s, 2 x Ac), 2.92 (6H, q, *J* 6.8, 3 x CH$_3$C*H*$_2$NH$^+$), 3.27 (3H, s,OCH$_3$), 3.57 (1H, dd, $J_{5,6a}$ 5.4, $J_{6a,6b}$ 11.8, H-6'^{a}), 3.70-3.75 (1H, m, H-5), 3.73 (1H, dd, $J_{5,6b}$ 2.5, H-6'b), 3.78 (1H, t $J_{3,4} = J_{4,5} = 9.6$, H-4'), 3.84 (1H, t $J_{3,4} = J_{4,5} = 9.7$, H-4), 3.94 (1H, dd, $J_{1,2}$ 1.8, H-2), 3.97 (1H, ddd, H-5'), 4.11 (1H, dd, $J_{1',2'}$ 2.0, H-2'), 4.12-4.15 (2H, m, H-6), 4.56 (1H, d, $J_{1,2}$ 1.7, H-1), 4.57, 4.59, 4.61 and 4.71 (4H, 4 x d, *J* 11.3, 2 x PhC*H*$_2$), 5.27 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.41 (1H, dd, $J_{2,3}$ 3.7, H-3'), 5.54 (1H, d, $J_{1,P}$ 7.2, H-1') and 7.21-7.31 (10H, m, Ph);

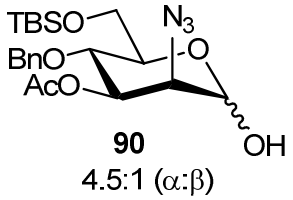
^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.81 (*C*H$_3$CH$_2$N), 20.67 and 20.71 (2 x *C*H$_3$CO), 45.59 (CH$_3$*C*H$_2$N), 55.07 (*C*H$_3$O), 61.75 (C-6'), 61.82 (C-2), 62.46 (d, $^3J_{C,P}$ 8.1, C-2'), 64.38 (br,

C-6), 71.07 (d, $^{3}J_{C,P}$ 12.5, C-5), 73.02, 73.18, 73.30 and 73.59 (C-3, C-3', C-4, C-4' and C-5'), 74.82 and 74.86 (2 x Ph*C*H_2), 94.04 (br, C-1'), 98.71 (C-1), 127.65-138.22 (Ph) and 170.02 (CH_3*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -2.69.

High resolution ES-MS(-): found *m/z*749.2218 $[M\text{-}Et_3N\text{-}H]^-$ ($C_{31}H_{38}N_6O_{14}P^-$ requires *m/z*749.2189).

3-*O*-Acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α,β-D-mannopyranose 90



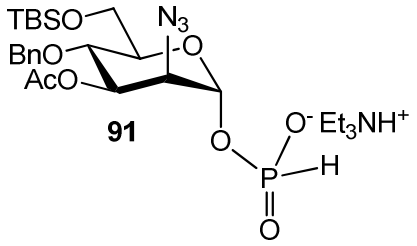
90
4.5:1 (α:β)

The thioglycoside **59** (237 mg, 478 μmol) was dissolved in dichloromethane (2 ml) and cooled to 0 ºC with stirring. Bromine (26 μl, 502 μmol, 1.05 eq) was then added, stirring was continued at 0 ºC for 30 min. The reaction mixture was then diluted with acetone-water (9:1; 10 ml) before the addition of silver carbonate (300 mg). Cooling was ceased and the mixture was stirred for an additional 16 h at room temperature. The suspension was then diluted with toluene, filtered through a Celite pad, and the filtrate was dried by filtration through cotton wool and concentrated. FCC [toluene-EtOAc, (9:1)] of the residue gave the hemiacetal derivative **90** (205 mg, 95%).

^{1}H NMR (500 MHz; $CDCl_3$): δ_H(signals of the α-anomer only) 0.00, 0.02 (6H, 2 x s, $(CH_3)_2Si$), 0.85 (9H, s, Me_3CSi), 1.99 (3H, s, Ac), 3.72 (1H, dd, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 11.3, H-6^a), 3.80 (1H, dd, $J_{5,6b}$ 3.5, H-6^b), 3.83 (1H, ddd, H-5), 3.88 (1H, t, $J_{3,4} = J_{4,5} = 9.0$, H-4), 3.98 (1H, dd, $J_{2,3}$ 3.7, H-2), 4.60 and 4.63 (2H, 2 x d, J 11.4, PhCH_2), 5.14 (1H, dd, $J_{1,2}$ 1.9, H-1), 5.36 (1H, dd, $J_{3,4}$ 9.0, H-3) and 7.07-7.30 (5H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C -5.32 (Me_2Si), 17.77 (Me_3C), 20.81 (CH_3CO), 25.94 (Me_3C) 61.92 (C-2), 62.24 (C-6), 72.83 (C-3, C-4 and C-5), 74.92 (PhCH_2), 92.77 (C-1), 127.75, 128.48 and 134.36 (Ph) and 170.00 (CH_3C=O).

Triethylammonium 3-*O*-acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl hydrogenphosphonate 91



The hemiacetal **90** (200 mg, 443 μmol) was dissolved in a mixture of dioxane (10 ml) and Et_3N (4.4 ml) and with stirring for 1 h. Salicylchlorophosphite (678 mg, 3.30 mmol, 7 eq) was then added and the mixture was stirred for 1 h. The reaction was then quenched with the addition of saturated aq. $NaHCO_3$ and stirring continued for additional 30 min. The mixture was then diluted with chloroform (80 ml) and the solution was washed successively with saturated aq. $NaHCO_3$, water, cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-

methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the H-phosphonate derivative **91** (269 mg, 91%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

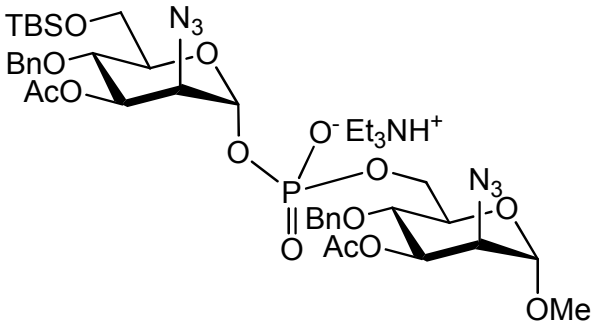
$[\alpha]_D$ +34.6 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 0.00, 0.03 (6H, 2 x s, Me_2Si), 0.86 (9H, s, Me_3CSi), 1.27 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$NH), 1.95 (3H, s, Ac), 3.00 (6H, q, *J* 6.8, 3 x CH$_3$C*H*$_2$NH), 3.73 (1H, m, H-6a), 3.81-3.87 (2H, m, H-6b and H-5), 3.98 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.9, H-4), 4.01 (1H, dd, $J_{1,2}$ 2.0, H-2), 4.61 and 4.65 (2H, 2 x d, *J* 11.4, PhC*H*$_2$), 5.35 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.54 (1H, dd, $J_{1,P}$ 8.6, H-1) 6.91 (1H, dd, $J_{P,H}$ 634, PH) and 7.19-7.30 (5H, m, Ph);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P 0.34.

High resolution ES-MS(-): found *m/z*514.1794 [M-Et_3N-H]$^-$ ($C_{21}H_{33}N_3O_8PSi^-$requires *m/z* 514.1780).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-[3-*O*-acetyl-2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl phosphate], triethylammonium salt 93



93

A mixture of the H-phosphonate **91** (269 mg, 436 μmol, 1.2 eq.) and compound **87** (128 mg, 363 μmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (107 μl, 872 μmol, 2.4 eq.) was added, and the solution was stirred for at 20 ^{o}C for 30 min. The solution was cooled to -40 ºC before a freshly prepared solution of iodine (221 mg, 872 μmol, 2.4 eq.) in a mixture pyridine-water (95:5; 1 ml) was added. The mixture was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$, cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the phosphodiester **93** (263 mg, 75%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2104 (-N_3);

$[\alpha]_D$ +29.6 (*c* 1, $CHCl_3$);

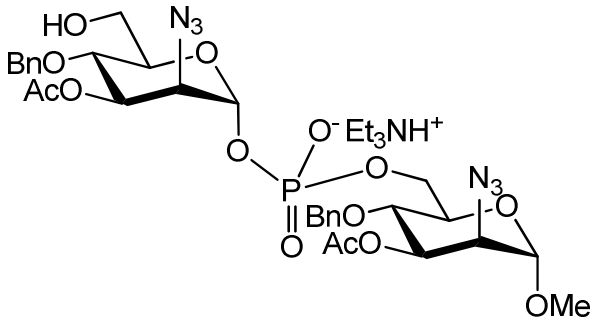
^{1}H NMR (500 MHz; $CDCl_3$): δ_H -0.04 and 0.00 (6H, 2 x s, Me_2Si), 0.83 (9H, s, Me_3CSi), 1.27 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$NH), 1.91 and 1.92 (6H, 2 x s, 2 x Ac), 2.87 (6H, q, *J* 6.8, 3 x CH$_3$C*H*$_2$NH), 3.25 (3H, s,OCH_3), 3.66 (1H, dd, $J_{5,6a}$ 1.4, $J_{6a,6b}$ 11.8, H-6'a), 3.69-3.72 (1H, m, H-5), 3.73 (1H, dd, $J_{5,6b}$ 2.6, H-6'b), 3.78 (1H, t $J_{3,4} = J_{4,5} = 9.8$, H-4), 3.79-3.84 (1H, m, H-5'), 3.92 (1H, dd, $J_{1,2}$ 1.7, H-2), 4.00 (1H, t, $J_{3,4} = J_{4,5} = 9.8$, H-4'), 4.11 (1H, dd, $J_{1',2'}$ 1.8, H-2'), 4.06-4.16 (2H, m, H-6), 4.53 (1H, d, H-1), 4.55, 4.57, 4.61 and 4.68 (4H, 4 x d, *J* 11.3, 2 x PhC*H*$_2$), 5.25 (1H, dd, $J_{2,3}$ 3.9, H-3), 5.39 (1H, dd, $J_{2',3'}$ 3.8, H-3'), 5.52 (1H, dd, $J_{1,P}$ 7.7, H-1') and 7.11-7.26 (10H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C -5.06 (Me_2Si), 8.81 (*C*H$_3$CH$_2$N), 17.77 (Me_3C), 20.68 and 20.77 (2 x *C*H$_3$CO), 25.92 (*Me*$_3$C), 45.60 (CH$_3$*C*H$_2$N), 54.96 (*C*H$_3$O), 61.73 (C-6'), 61.82 (C-2), 62.44 (d, $^{3}J_{C,P}$ 6.3, C-2'), 64.42 (d, $^{2}J_{C,P}$ 4.4, C-6), 71.12 (d, $^{3}J_{C,P}$ 8.8, C-5), 72.42 (C-5') 73.07, 73.21, 73.43 and 73.65 (C-3, C-3', C-4 and C-4'), 74.78 and 74.95 (2 x Ph*C*H$_2$), 94.24 (d, $^{2}J_{C,P}$ 4.3, C-1'), 98.60 (C-1), 127.54-128.44 (Ph) and 170.02 (CH$_3$*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -2.74.

High resolution ES-MS(-): found *m/z*863.3069 [M-Et$_3$N-H]$^-$ ($C_{37}H_{52}N_6O_{14}PSi^-$ requires *m/z* 863.3054).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate), triethylammonium salt 92



92

The disaccharide phosphate **93** (258 mg, 267 μmol) was dissolved in THF (5 ml) with stirring. To this $Et_3N{\cdot}3HF$ (218 μl, 1.34 mmol, 5 eq) was added and the solution was stirred at ambient temperature for 16 h. The solvent was then removed under reduced pressure. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→90:10:1)] of the residue gave the phosphodiester **92** (216 mg, 95%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +23.3 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.20 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$NH), 1.94 and 1.96 (6H, 2 x s, 2 x Ac), 2.92 (6H, q, *J* 6.8, 3 x CH$_3$C*H*$_2$NH), 3.27 (3H, s,OCH$_3$), 3.57 (1H, dd, $J_{5,6a}$ 5.4, $J_{6a,6b}$ 11.8, H-6'[a]), 3.70-3.75 (1H, m, H-5), 3.73 (1H, dd, $J_{5,6b}$ 2.5, H-6'b), 3.78 (1H, t $J_{3,4} = J_{4,5} = 9.6$, H-4'), 3.84 (1H, t $J_{3,4} = J_{4,5} = 9.7$, H-4), 3.94 (1H, dd, $J_{1,2}$ 1.8, H-2), 3.97 (1H, ddd, H-5'), 4.11 (1H, dd, $J_{1',2'}$ 2.0, H-2'), 4.12-4.15 (2H, m, H-6), 4.56 (1H, d, $J_{1,2}$ 1.7, H-1), 4.57, 4.59, 4.61 and 4.71 (4H, 4 x d, *J* 11.3, 2 x PhC*H*$_2$), 5.27 (1H,

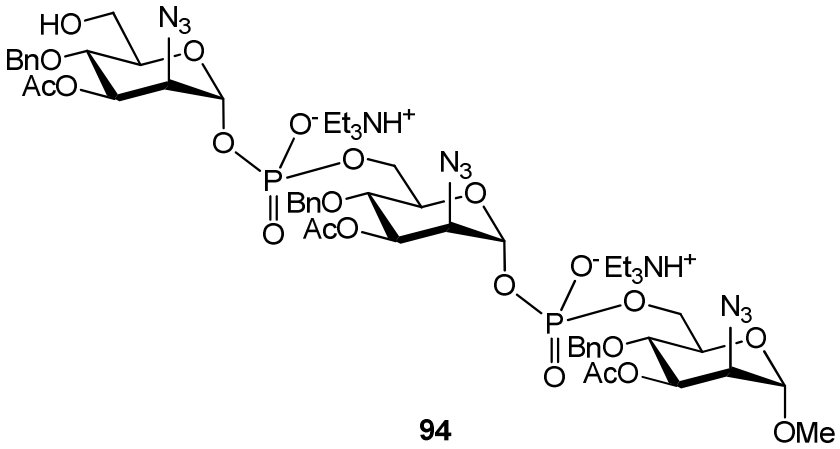
dd, $J_{2,3}$ 3.8, H-3), 5.41 (1H, dd, $J_{2,3}$ 3.7, H-3'), 5.54 (1H, d, $J_{1,P}$ 7.2, H-1') and 7.21-7.31 (10H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.81 (CH_3CH_2N), 20.67 and 20.71 (2 x CH_3CO), 45.59 (CH_3CH_2N), 55.07 (CH_3O), 61.75 (C-6'), 61.82 (C-2), 62.46 (d, $^3J_{C,P}$ 8.1, C-2'), 64.38 (br, C-6), 71.07 (d, $^3J_{C,P}$ 12.5, C-5), 73.02, 73.18, 73.30 and 73.59 (C-3, C-3', C-4, C-4' and C-5'), 74.82 and 74.86 (2 x $PhCH_2$), 94.04 (br, C-1'), 98.71 (C-1), 127.65-138.22 (Ph) and 170.02 (CH_3C=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -2.69.

High resolution ES-MS(-): found *m/z*749.2218 $[M\text{-}Et_3N\text{-}H]^-$ ($C_{31}H_{38}N_6O_{14}P^-$ requires *m/z*749.2189).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-[3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt 94



94

A mixture of the H-phosphonate **89** (110 mg, 137 μmol, 1.3 eq.) and the disaccharide phosphate **92** (90 mg, 106 μmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (22 μl, 180 μmol, 1.7 eq.) was added, and the solution was stirred for at 20 ºC for 30 min. The solution was cooled to -40 ºC before Et_3N (44 μl, 360 μmol, 3.4 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (46 mg, 180 μmol, 1.7 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, prior it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. The residuewas dissolved in CH_2Cl_2 (5 ml) and cooled to -10 ºC with stirring. A mixture of DCM-TFA (49:1, 5 ml) was then prepared and cooled to -10 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition ofsaturated aq. $NaHCO_3$. The solution was then diluted with chloroform and washed successively with saturated aq. $NaHCO_3$ and cold 0.5 M TEAB buffer, by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→85:15:1)] of the residue gave the phosphosaccharide **94** (130 mg, 74%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +13.8 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.12 (18H, t, *J* 7.3, 6 x C*H*$_3$CH$_2$N), 1.92, 1.93 and 1.95 (9H, 3 x s, 3 x Ac), 2.76 (12H, br, 6 x CH$_3$C*H*$_2$N), 3.27 (3H, s,OCH$_3$), 3.58 (1H, m, H-6$^{a''}$), 3.70-3.85 (5H, m, H-4, H-4', H-4'', H-5, and H-6$^{b''}$), 3.89-3.92 (1H, m, H-5''), 3.93 (1H, dd, $J_{1,2}$ 1.6, H-2), 4.01-4.23 (7H, m, H-2', H-2'', H-5', H-6^a, H-6^b, H-6$^{a'}$ and H-6$^{b'}$), 4.54-4.61 (5H, m, 2 x PhC*H*$_2$ and H-1),4.67-4.74 (2H, m, PhC*H*$_2$), 5.26 (1H, dd,

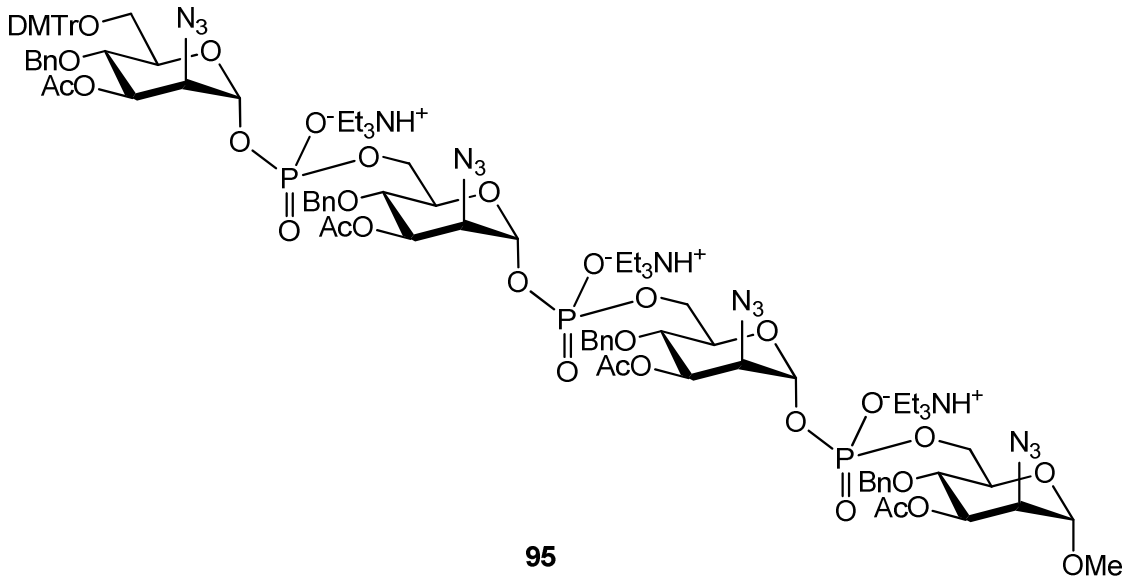
$J_{2,3}$ 3.8, H-3), 5.37 (1H, m, H-3''), 5.43-5.53 (3H, m, H-1', H-1'' and H-3') and 7.14-7.27 (15H, m, 3 x Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 9.44 (CH_3CH_2N), 20.55, 20.67 and 20.78 (3 x CH_3CO), 45.73 (CH_3CH_2N), 55.16 (CH_3O), 61.52 (C-6''), 61.83 (C-2), 62.52 (2 x d, $^3J_{C,P}$ 7.3, $^3J_{C,P}$ 7.6, C-2', C-2''), 64.32 and 64.40 (br and d, $^2J_{C,P}$ 5.4, C-6 and C-6'), 71.13 (d, $^3J_{C,P}$ 8.5, C-5), 72.41 (d, $^3J_{C,P}$ 7.1, C-5'), 72.46, 72.90, 72.99, 73.07, 73.13 and 73.62 (C-3, C-3', C-3'', C-4, C-4', C-4'' and C-5''), 74.66, 74.80 and 74.92 (3 x $PhCH_2$), 93.98 and 94.26 (2 x br, C-1' and C-1''), 98.55 (C-1), 126.46-137.46 (Ph), 169.67, 169.76 and 170.02 (CH_3C=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.18 and -2.77.

High resolution ES-MS(-): found *m/z*1148.2894 $[M\text{-}2Et_3N\text{-}H]^-$ ($C_{46}H_{56}N_9O_{22}P_2^-$ requires *m/z*1148.3021).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-{3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-[3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-(*p,p'*-dimethoxytrityl)-α-D-mannopyranosyl phosphate]}), tris-triethylammonium salt 95



95

A mixture of the H-phosphonate **89** (93 mg, 116 µmol, 2 eq.) and the trisaccharide diphosphate **94** (79 mg, 58 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (19 µl, 151 µmol, 2.6 eq.) was added, and the solution was stirred for at 20 ^{o}C for 30 min. The solution was cooled to -40 ºC prior Et_3N (50 µl, 302 µmol, 5.2 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (38 mg, 151 µmol, 2.6 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, before it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→80:20:1)] of the residue gave the phosphosaccharide **95** (91 mg, 73%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +12.8 (*c* 1, $CHCl_3$);

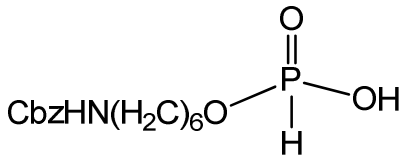
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.05 (27H, t, *J* 7.3, 9 x C*H*$_3$CH$_2$N), 1.87, 1.92, 1.93 and 1.95 (12H, 4 x s, 4 x Ac), 2.65 (18H, br, 9 x CH$_3$C*H*$_2$N), 3.04 (1H, dd, $J_{5''',6a'''}$ 2.2, $J_{6a''',6b'''}$ 10.3, H-6^{a}'''), 3.26 (3H, s,OCH_3), 3.40 (1H, br d, H-6^{b}'''), 3.67 (6H, 2 x s,2 x OCH_3), 3.71 (1H, br d, H-5), 3.77 (1H, t, $J_{3,4} = J_{4,5} = 9.6$, H-4'), 3.81 (1H, t, $J_{3,4} = J_{4,5} =$ 9.6, H-4), 3.86-4.02 (6H, m, H-2, H-4'', H-5'- H-5''', H-6^{a}), 4.03-4.23 (10H, m, H-2'- H-2''', H-4''', H-6^{b}, H-6^{a}', H-6^{b}', H-6^{a}'', H-6^{b}'', PhC*H*$_2$), 4.35 (1H, d, *J*11.0, PhC*H*$_2$), 4.48 (1H, d, *J*11.0, PhC*H*$_2$) 4.54-4.77 (7H, m, 3 x PhC*H*$_2$ and H-1), 5.26 (1H, dd, $J_{2,3}$ 3.8, $J_{3,4}$ 9.4, H-3), 5.37-5.43 (3H, m, H-1'', H-3' and H-3'''), 5.44-5.49 (2H, m, H-1' and H-3''), 5.68 (1H, br d, $J_{1''',P}$ 6.9, H-1''') and 6.64-7.48 (33H, m, 4 x Ph, C_6H_4);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 10.32 (*C*H$_3$CH$_2$N), 20.60, 20.67 and 20.78 (*C*H$_3$CO), 45.96 (*C*H$_3$CH$_2$N), 55.01 (CH_3O), 55.16 (CH_3O), 61.52 (C-6'''), 61.83 (C-2), 62.53 (br, C-2', C-2'' and C2'''), 64.23 (br, C-6, C-6' and C-6'''), 71.06 (d, $^{3}J_{C,P}$ 7.6, C-5), 72.22 (2 x d, $^{3}J_{C,P}$ 7.2, $^{3}J_{C,P}$ 8.1, C-5' and C-5''), 72.45 (C-5'''), 72.87, 73.02 and 73.59 (C-3 - C-3''' and C-4 - C-4'''), 74.66, 74.82 and 74.91 (Ph*C*H$_2$), 85.58 (Ar$_3$*C*), 93.97 (br, C-1' and C-1''), 94.26 (br, C-1'''), 98.55 (C-1), 113.06-158.37 (Ph, C_6H_4) and 169.71 and 169.77 (*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.28, -3.18 and -3.06.

High resolution ES-MS(-): found *m/z*1849.5724 [M-3Et$_3$N-H]$^-$ ($C_{82}H_{92}N_{12}O_{32}P_3^-$ requires *m/z*1849.5159), 1871.5583 [M-3Et$_3$N-2H+Na]$^-$ ($C_{82}H_{91}N_{12}NaO_{32}P_3^-$ requires *m/z*1871.4978), 1887.5346 [M-3Et$_3$N-2H+K]$^-$ ($C_{82}H_{91}KN_{12}O_{32}P_3^-$ requires *m/z*1887.4718), 1893.5346 [M-3Et$_3$N-3H+2Na]$^-$ ($C_{82}H_{90}N_{12}Na_2O_{32}P_3^-$ requires *m/z*1893.4798).

Triethylammonium 6-(N-benzyloxycarbonyl)aminohexyl hydrogenphosphonate 97



97

To a solution of 6-(*N*-benzyloxycarbonyl)aminohexanol (300 mg, 1.20 mmol) in MeCN (20 ml) was added PCl_3 (794 µl, 9 mmol). The reaction was stirred at ambient temperature for 30 min before evaporating to dryness. The residue was dissolved in MeCN (10 ml) before the dropwise addition of 0.5 M TEAB buffer (5 ml). The solution was allowed to stir for an additional 30 min before removing the solvent under reduced pressure to give a white solid. This was then dissolved in $CHCl_3$ (1 ml) before ether (20 ml) was added. The white precipitate which formed was filtered off. The solvent was then removed under reduced pressure to give the H-phosphonate **97** (440 mg, 88%) as an amorphous solid.

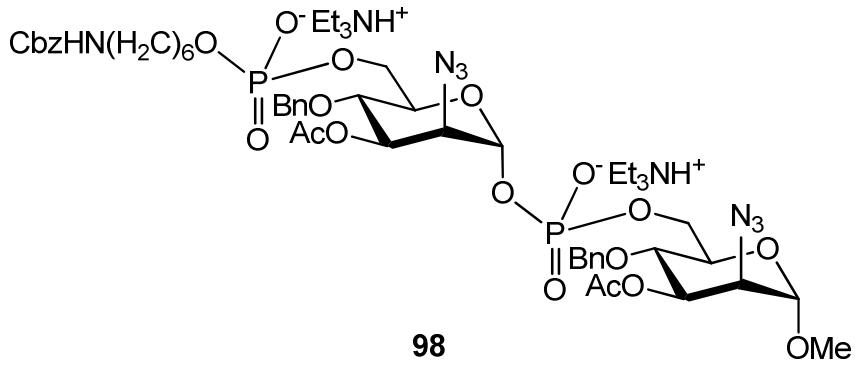
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.25 (9H, t, *J* 7.3, 3 x C*H*$_3$CH$_2$N), 1.26-1.35 (4H, m, 2 x CH_2), 1.42 (2H, m, NCH$_2$C*H*$_2$), 1.56 (2H, m, OCH$_2$C*H*$_2$), 2.98 (6H, dq, $J_{CH2,NH}$ 3.6, 3 x CH$_3$C*H*$_2$N), 3.11 (2H, m, CH$_2$N), 3.80 (2H, dt, $J_{H,H}$ 6.6, $J_{H,P}$ 7.8, CH$_2$OP), 4.91 (1H, br, NH), 5.02 (2H, s, PhC*H*$_2$O), 6.76 (1H, d, $J_{H,P}$ 624.4, HP), 7.22-7.30 (5H, m, Ph) and 12.57 (1H, br, Et$_3$N*H*$^+$);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.53 (*C*H$_3$CH$_2$N), 25.38, 26.24, 29.85 (3 x CH_2), 30.57 (d, $^3J_{C,P}$ 6.6, *C*H$_2$CH$_2$OP), 40.91 (CH$_2$*C*H$_2$N), 45.38 (CH$_3$*C*H$_2$N), 63.74 (d, $^2J_{C,P}$ 4.8, CH$_2$OP), 66.52 (Ph*C*H$_2$) and 128.06, 128.14, 128.52 (Ph);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P 4.95.

High resolution ES-MS(-): found *m/z*314.1183 $[M\text{-}Et_3N\text{-}H]^-$ ($C_{14}H_{21}NO_5P^-$ requires *m/z*314.1163).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-{3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-[6-(benzyloxycarbonyl)aminohexyl phosphate]}, bis-triethylammonium salt 98



98

A mixture of the H-phosphonate **97** (21 mg, 51 µmol, 1.3 eq.) and the disaccharide phosphate **92** (33 mg, 39 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (9 µl, 76 µmol, 1.9 eq.) was added, and the solution was stirred for at 20 ^oC for 30 min. The solution was cooled to -40 ºC prior Et_3N (42 µl, 304 µmol, 7.8 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (40 mg, 152 µmol, 3.9 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, before it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and

concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→85:15:1)] of the residue gave the phosphosaccharide **98** (43 mg, 88%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +27.3 (*c* 1, $CHCl_3$);

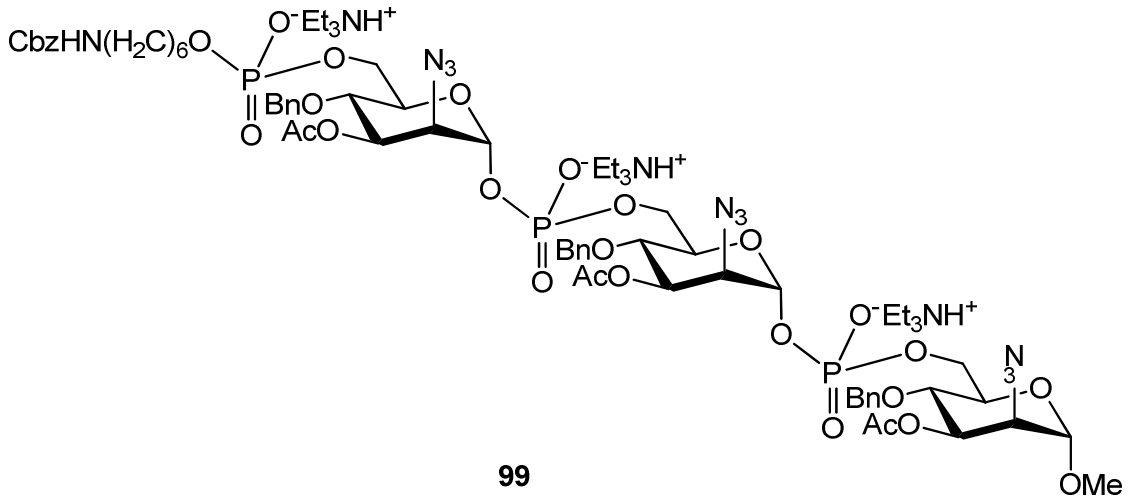
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.22 (18H, t, *J* 7.3, 6 x C*H*$_3$CH$_2$N), 1.24-1.57 (8H, m, 4 x CH_2), 1.93 and 1.94 (6H, 2 x s, 2 x Ac), 2.94 (12H, q, 6 x CH_3C*H*$_2$N), 3.04-3.13 (2H, m, NCH_2), 3.26 (3H, s,OCH_3), 3.70 (1H, br d, H-5), 3.73-3.81 (2H, m, CH_2OP), 3.82 (1H, t, $J_{3,4} = J_{4,5} = 9.7$, H-4), 3.88 (1H, t, $J_{3',4'} = J_{4',5'} = 9.6$, H-4'), 3.93 (1H, dd, $J_{1,2}$ 1.6, H-2), 3.95-4.02 (2H, m,H-5', H-6$^{a'}$), 4.06-4.13 (3H, m,H-6^a, H-6^b and H-6$^{b'}$), 4.21 (1H, m, H-2'), 4.55 (1H, d, H-1), 4.57, 4.59, 4.70 and 4.72 (4H, 4 x d, *J* 11.3, 2 x PhC*H*$_2$), 4.99-5.06 (2H, m, PhC*H*$_2$OCO), 5.26 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.36 (1H, m, NH), 5.44-5.50 (2H, m, H-1' and H-3') and 7.15-7.7.29 (15H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.57 (*C*H$_3$CH$_2$N), 20.66 and 20.67 (*C*H$_3$CO), 25.27 (CH_2), 26.21 (CH_2), 29.77 (CH_2), 30.59 (d, $^3J_{C,P}$ 7.1,*C*H$_2$CH$_2$OP), 40.85 (CH_2N), 45.50 (CH$_3$*C*H$_2$N), 55.00 (CH_3O), 61.77 (C-2), 61.42 (d, $^3J_{C,P}$ 6.3, C-2'), 63.36 (d, $^2J_{C,P}$ 6.4, CH_2OP), 64.18 (d, $^2J_{C,P}$ 5.1, C-6'), 65.15 (d, $^2J_{C,P}$ 5.7, C-6), 66.31 (Ph*C*H$_2$OCO), 71.02 (d, $^3J_{C,P}$ 8.9, C-5), 72.29 (d, $^3J_{C,P}$ 8.0, C-5'), 73.00, 73.02, 73.04 and 73.55 (C-3, C-3', C-4, C-4'), 74.75 and 74.90 (Ph*C*H$_2$), 93.89 (d, $^2J_{C,P}$ 4.8, C-1'), 98.50 (C-1), 127.46-138.31 (Ph), 156.51 (HNC=O) and 169.77 and 170.04 (CH$_3$*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.10 (P) and 0.23 (P').

High resolution ES-MS(-): found *m/z*1062.3274 [M-2Et_3N-H]$^-$ ($C_{45}H_{58}N_7O_{19}P_2^-$requires *m/z*1062.3268).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-{3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-[6-(benzyloxycarbonyl)aminohexyl phosphate]}), tris-triethylammonium salt 99



99

A mixture of the H-phosphonate **97** (25 mg, 58 µmol, 1.2 eq.) and the trisaccharide diphosphate **94** (66 mg, 49 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (9 µl, 76 µmol, 1.55 eq.) was added, and the solution was stirred for at 20 ^{o}C for 30 min. The solution was cooled to -40 ºC prior Et_3N (21 µl, 147 µmol, 3 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (19 mg, 74 µmol, 1.5 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, before it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→80:20:1)] of the residue gave the phosphosaccharide **99** (43 mg, 88%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2103 ($-N_3$);

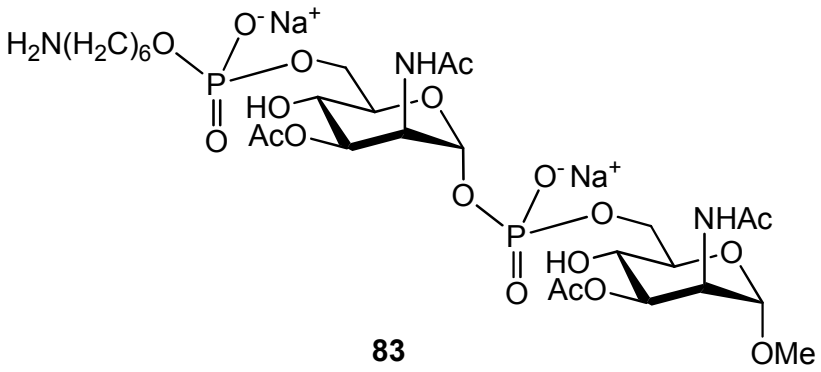
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.21 (27H, t, *J*7.3, 9 x C*H*$_3$CH$_2$N), 1.22-1.29 (4H, m, 2 x CH_2), 1.33-1.41 (2H, m, NCH$_2$C*H*$_2$), 1.46-1.54 (2H, m, POCH$_2$C*H*$_2$), 1.93 and 1.94 (9H, 2 x s, 3 x Ac), 2.95 (18H, q, 9 x CH$_3$C*H*$_2$N), 3.03-3.10 (2H, m, NCH$_2$), 3.26 (3H, s,OCH$_3$), 3.70-4.16 (14H, m, H-4, H-4', H-4'', H-5, H-5', H-5'', H-6^a, H-6^b, H-6$^{a'}$, H-6$^{b'}$, H-6$^{a''}$, H-6$^{b''}$ and CH$_2$OP), 3.92 (1H, dd, $J_{1,2}$ 1.6, H-2), 3.94 (1H, dd, $J_{1',2'}$ 2.0, H-2'), 4.20 (1H, br, H-2''), 4.55 (1H, d, H-1), 4.57, 4.59, 4.71 and 4.74 (6H, 4 x d, *J*11.3, 3 x PhC*H*$_2$), 4.99-5.06 (2H, m, PhC*H*$_2$OCO), 5.23 (1H, m, NH), 5.26 (1H, dd, $J_{2,3}$ 3.8, H-3), 5.39-5.50 (4H, m, H-1', H-1'', H-3' and H-3'') and 7.13-7.29 (20H, m, Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.55 (*C*H$_3$CH$_2$N), 20.64 and 20.70 (*C*H$_3$CO), 25.29 (CH_2), 26.25 (CH_2), 29.79 (CH_2), 30.63 (br,*C*H$_2$CH$_2$OP), 40.92 (CH$_2$N), 45.51 (CH$_3$*C*H$_2$N), 55.05 (CH$_3$O), 61.81 (C-2), 62.49 (d, $^3J_{C,P}$ 7.3, C-2' and C-2''), 63.65 (d, $^2J_{C,P}$ 3.6, CH$_2$OP), 64.24 (d, $^2J_{C,P}$ 3.6), 64.36 (d, $^2J_{C,P}$ 4.8) (C-6' and C-6''), 65.34 (d, $^2J_{C,P}$ 4.8, C-6), 66.40 (Ph*C*H$_2$OCO), 70.99 (d, $^3J_{C,P}$ 7.7, C-5), 72.08 (d, $^3J_{C,P}$ 8.5), 72.21 (d, $^3J_{C,P}$ 7.4) (C-5' and C-5''), 72.84, 73.04, 73.19 and 73.61 (C-3, C-3', C-3'', C-4, C-4' and C-4''), 74.85 and 74.95 (Ph*C*H$_2$), 94.00 (d, $^2J_{C,P}$ 3.8, C-1' and C-1''), 98.53 (C-1), 127.59-138.45 (Ph), 156.50 (HNC=O) and 169.80 and 170.06 (CH$_3$*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.03 (P and P') and 0.51 (P").

High resolution ES-MS(-): found *m/z*1461.4125 [M-3Et$_3$N-H]$^-$ ($C_{60}H_{76}N_{10}O_{27}P_3^-$ requires *m/z*1461.4100).

Methyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranoside 6-[2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate 6-(6-aminohexyl phosphate)], bis-sodium salt 83



83

A solution of the phosphosaccharide **98** (100mg, 79 μmol) in a mixture of THF (100 μl) and MeOH (2 ml) was prepared and cooled to 0 ºC, after which $NiCl_2.6H_2O$ (20 mg) was added. Over the course of 1 h, $NaBH_4$ (4 x 15 mg) was added while stirring at 0 ºC. After this time Ac_2O (500 μl) was added and the solution stirred for an additional 30 min while warming to ambient temperature. The solution was then evaporated to dryness, the residue was taken in $CHCl_3$ and the solution was washed with 0.5 M TEAB buffer. The organic layer was then dried by filtration through cotton wool before removing solvent under reduced pressure. The residue was then dissolved in MeOH (5 ml) and treated with IR-120 (Na^+) resin while stirring for 5 h. The resin was then filtered off and the solution evaporated to dryness. The residue was then dissolved in a mixture of THF (0.5 ml) and MeOH (4 ml) with the addition of AcOH (15 μl). The solution was then hydrogenated over $Pd(OH)_2/C$ (70 mg) at ambient temperature for 3 h. The mixture was then filtered through a Celite pad, the filtrate was concentrated and the residue was freeze-dried (from an aqueous solution) to give the phosphosaccharide **83** (50 mg, 96%) as a white solid.

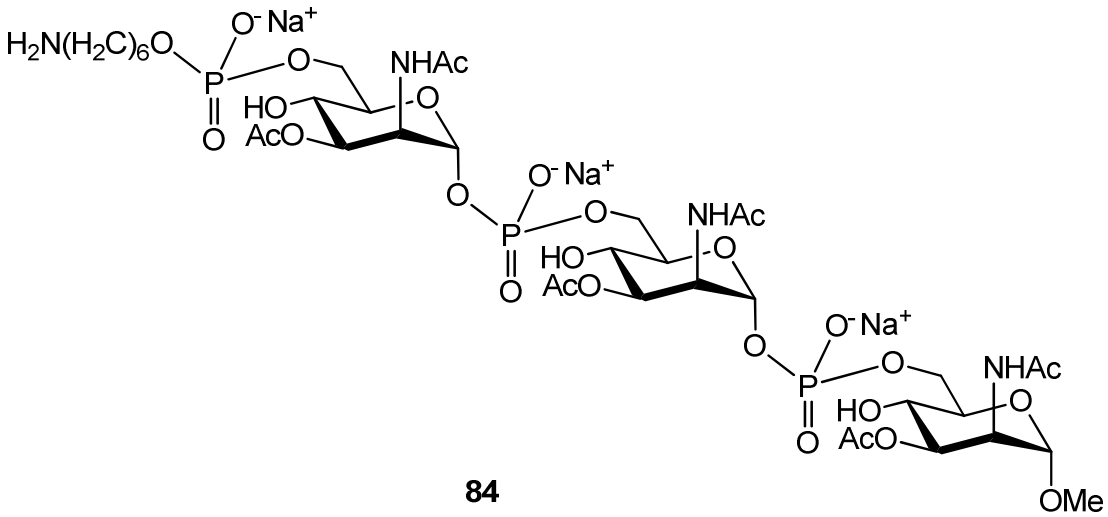
^{1}H NMR (500 MHz; D_2O): δ_H 1.29 (4H, m, 2 x CH_2), 1.54 (4H, m, C*H*$_2$CH_2OP and C*H*$_2$CH_2N), 1.91, 1.94 and 1.95 (12H, 3 x s, 4 x Ac), 2.87 (2H, t, *J* 7.4, NCH_2), 3.29 (3H, s,OCH_3), 3.70-3.82 (4H, m, H-4, H-5 and CH_2OP), 3.88 (1H, t, $J_{3,4} = J_{4,5} = 10.0$, H-4'), 3.93-4.01 (2H, m,H-5', H-6$^{a'}$), 4.03-4.12 (3H, m,H-6^{a}, H-6^{b} and H-6$^{b'}$), 4.37 (1H, br d, H-2), 4.45 (1H, br d, H-2'), 4.59 (1H, br, H-1), 4.91 (1H, dd, $J_{2,3}$ 4.4, $J_{3,4}$ 8.6, H-3), 5.06 (1H, dd, $J_{2,3}$ 4.3, $J_{3,4}$ 10.0, H-3) and 5.33 (1H, br d, $J_{1,P}$ 7.4, H-1');

^{13}C NMR (125 MHz; D_2O): δ_C 20.27 (*C*H_3COO), 21.62 (*C*H_3CON), 24.38 (CH_2), 25.10 (CH_2), 26.53 (CH_2), 29.40 (d, $^{3}J_{C,P}$ 7.1,*C*H_2CH_2OP), 39.33 (CH_2N), 49.85 (C-2), 50.66 (d, $^{3}J_{C,P}$ 7.3, C-2'), 54.82 (CH_3O), 63.22 and 63.63 (C-4 and C-4'), 63.72 (br, C-6), 64.44 (d, $^{2}J_{C,P}$ 4.1, C-6'), 66.22 (d, $^{2}J_{C,P}$ 5.0, CH_2OP), 70.88 (d, $^{3}J_{C,P}$ 6.9, C-5), 71.73 and 72.31 (C-3 and C-3'), 72.15 (d, $^{3}J_{C,P}$ 12.3, C-5'), 94.87 (d, $^{2}J_{C,P}$ 3.7, C-1'), 99.39 (C-1), 173.37 (CH_3*C*OO) and 174.40 (CH_3*C*ON);

^{31}P NMR (200 MHz; D_2O): δ_P -2.52 (P) and 0.80 (P').

High resolution ES-MS(-): found *m/z*780.2301 $[M - 2Na + H]^-$ ($C_{27}H_{48}N_3O_{19}P_2^-$requires *m/z*780.2363).

Methyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranoside 6-{2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate 6-[2-acetamido-3-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate 6-(6-aminohexyl phosphate)]}, tris-sodium salt 84



84

A solution of the phosphosaccharide **99** (40 mg, 22.6 μmol) in a mixture of THF (100 μl) and MeOH (2 ml) was prepared and cooled to 0 ºC, after which $NiCl_2 \cdot 6H_2O$ (20 mg) was added. Over the course of 1 h, $NaBH_4$ (4 x 15 mg) was added while stirring at 0 ºC. After this time Ac_2O (500 μl) was added and the solution stirred for an additional 30 min while warming to ambient temperature. The solution was then evaporated to dryness, the residue was taken in $CHCl_3$ and the solution was washed with 0.5 M TEAB buffer. The organic layer was then dried by filtration through cotton wool before removing solvent under reduced pressure. The residue was then dissolved in MeOH (5 ml) and treated with IR-120 (Na^+) resin while stirring for 5 h. The resin was then filtered off and the solution evaporated to dryness. The residue was then dissolved in a mixture of THF (0.5 ml) and MeOH (3 ml) with the addition of AcOH (10 μl). The solution was then hydrogenated over $Pd(OH)_2/C$ (30 mg) at ambient temperature for 3 h. The mixture was then filtered through a Celite pad, the filtrate was concentrated and

the residue was freeze-dried (from an aqueous solution) to give the phosphosaccharide **84** (24 mg, 90%) as a white solid.

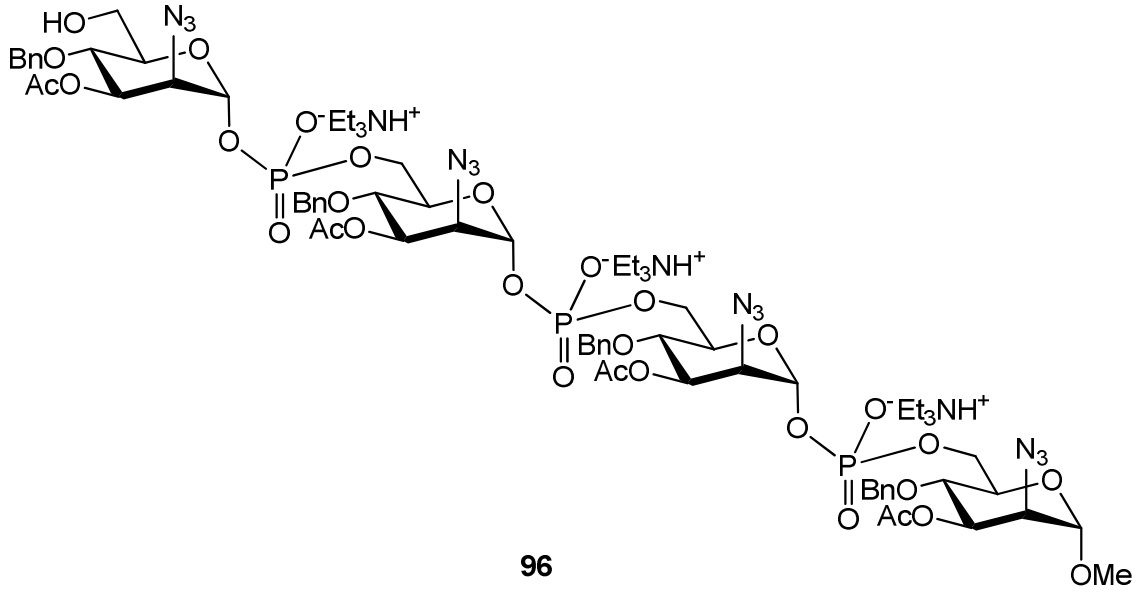
^{1}H NMR (500 MHz; D_2O): δ_H 1.30 (4H, m, 2 x CH_2), 1.55 (4H, m, C*H*$_2$CH$_2$OP and C*H*$_2$CH$_2$N), 1.92 (9H, m, 3 x Ac), 1.94 (3H, s, Ac), 1.96 (6H, s, 2 x Ac), 2.88 (2H, t, *J* 7.4, NCH_2), 3.30 (3H, s,OCH_3), 3.74-3.92 (6H, m, H-4, H-4', H-4'', H-5 and CH_2OP), 3.92-4.16 (8H, m, H-5', H-5'', H-6^{a}, H-6^{b}, H-6$^{a'}$, H-6$^{b'}$, H-6$^{a''}$, H-6$^{b''}$), 4.38 (1H, dd, $J_{1,2}$ 1.2, $J_{2,3}$ 4.3, H-2), 4.44 (1H, dd, $J_{1,2}$ 1.6, $J_{2,3}$ 4.0, H-2''), 4.46 (1H, dd, $J_{1,2}$ 1.6, $J_{2,3}$ 4.0, H-2'), 4.60 (1H, d, $J_{1,2}$ 1.2, H-1), 4.91 (1H, dd, $J_{2,3}$ 4.5, $J_{3,4}$ 9.5, H-3), 5.03-5.09 (2H, m, H-3' and H-3'') and 5.28-5.35 (2H, m, H-1' and H-1'');

^{13}C NMR (125 MHz; D_2O): δ_C 20.28 (*C*H$_3$COO), 21.64 (*C*H$_3$CON), 24.39 (CH_2), 25.09 (CH_2), 26.53 (CH_2), 29.41 (d, $^{3}J_{C,P}$ 6.7,*C*H$_2$CH$_2$OP), 39.35 (CH_2N), 49.87 (C-2), 50.62 (d, $^{3}J_{C,P}$ 8.7) and 50.73 (d, $^{3}J_{C,P}$ 6.5) (C-2' and C-2''), 54.83 (CH_3O), 63.24 (br, C-4, C-4' and C-4''), 63.73 (br, C-6 and C-6'), 64.43 (br, C-6''), 66.17 (br, CH_2OP), 70.92 (br), 71.55 (br) and 72.15 (br) (C-5, C-5' and C-5''), 71.72 and 72.35 (2C) (C-3, C-3' and C-3''), 94.82 (2C, d, $^{2}J_{C,P}$ 4.6, C-1' and C-1''), 99.39 (C-1), 173.36 (CH$_3$*C*OO) and 174.42 (CH$_3$*C*ON);

^{31}P NMR (200 MHz; D_2O): δ_P -2.54, -2.33 (P and P') and 0.81 (P'').

High resolution ES-MS(-): found *m/z* 552.1499 $[M - 3Na + H]^{2-}$ ($C_{37}H_{63}N_4O_{28}P_3^{2-}$ requires *m/z*552.14265), 1063.2770 $[M - CH_3CO - 3Na + 3H]^{-}$ ($C_{35}H_{62}N_4O_{27}P_3^{-}$requires *m/z*1063.2820), 1085.2603 $[M - CH_3CO - 2Na + 2H]^{-}$ ($C_{35}H_{61}N_4NaO_{27}P_3^{-}$requires *m/z*1085.2639), 1105.2905 $[M - 3Na + 2H]^{-}$ ($C_{37}H_{64}N_4O_{28}P_3^{-}$requires *m/z*1105.2925), 1127.2760 $[M - 2Na + H]^{-}$ ($C_{37}H_{63}N_4NaO_{28}P_3^{-}$requires *m/z*1127.2745).

Methyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranoside 6-{3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-[3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-mannopyranosyl phosphate 6-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-α-D-mannopyranosyl phosphate)]}, tris-triethylammonium salt 96



The tetrasaccharide triphosphate **95** (84 mg, 39 μmol) was dissolved in CH_2Cl_2 (5 ml) and cooled to -10 ºC with stirring. A mixture of CH_2Cl_2-TFA (49:1, 5 ml) was then prepared and cooled to -10 ºC before adding to the stirring solution. After 1 min the reaction was quenched with the addition of saturated aq. $NaHCO_3$. The mixture was then diluted with $CHCl_3$ (60 ml), and the solution was washed successively with saturated aq. $NaHCO_3$ and 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→80:20:1)] of the residue gave the phosphosaccharide **96** (66 mg, 91%).

υ_{max} (film)/cm^{-1} (*inter alia*): 2103 (-N_3);

$[\alpha]_D$ +15.3 (*c* 1, $CHCl_3$);

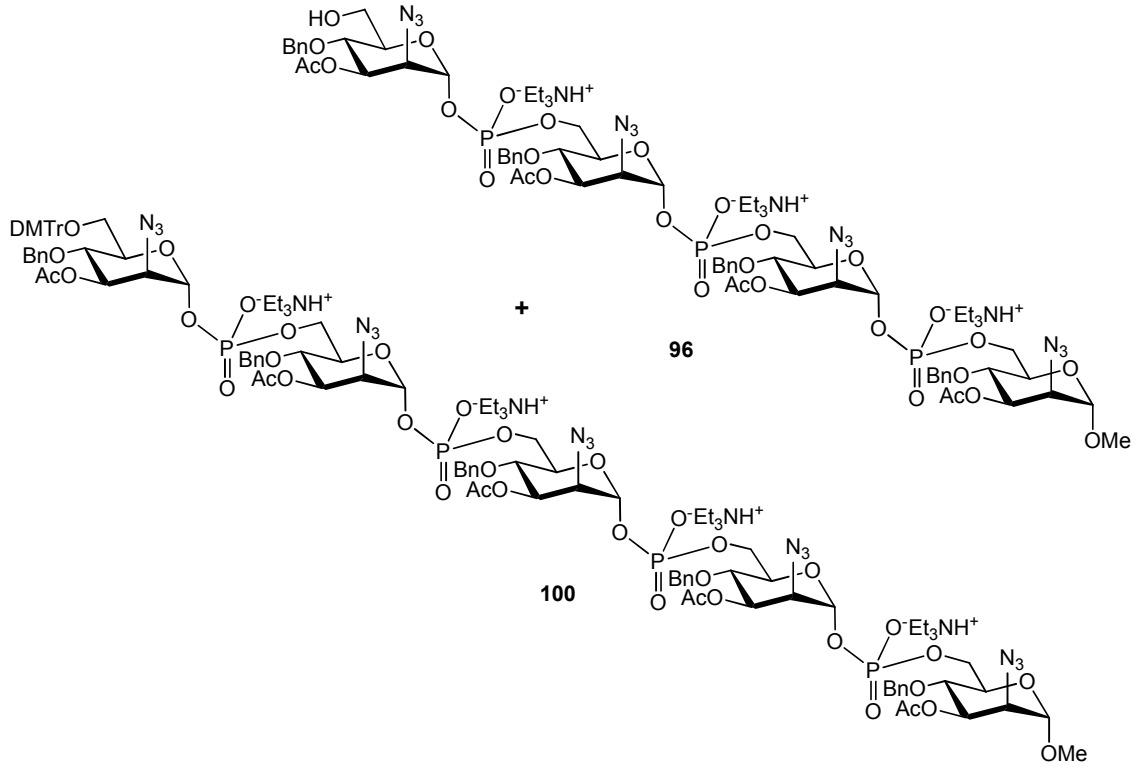
^{1}H NMR (500 MHz; $CDCl_3$): δ_H 1.18 (27H, t, *J* 7.3, 9 x C*H*$_3$CH$_2$N), 1.98, 1.99 and 2.01 (12H, 3 x s, 4 x Ac), 2.84 (18H, br, 9 x CH$_3$C*H*$_2$N), 3.33 (3H, s,OCH$_3$), 3.68 (1H, dd, $J_{5,6a'''}$ 4.7, $J_{6a''',6b'''}$ 11.9, H-6$^{a'''}$), 3.78-3.83 (1H, m,H-5 and H-6$^{b'''}$), 3.87 (2H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6, , $J_{3''',4'''}$ = $J_{4''',5'''}$ = 9.6, H-4 and H-4'''), 3.94-4.04 (5H, m, H-2, H-4', H-4'', H-5', H-5'''), 4.08 (1H, br d, $J_{4'',5''}$ 9.1, H-5''), 4.13-4.23 (8H, m, H-2', H-2''', H-6^{a}, H-6^{b}, H-6$^{a'}$, H-6$^{b'}$, H-6$^{a''}$, H-6$^{b''}$), 4.29 (1H, br, H-2''), 4.61-4.68 (6H, m, PhC*H*$_2$ and H-1) 4.76-4.84 (3H, m, PhC*H*$_2$), 5.33 (1H, dd, $J_{2,3}$ 3.8, $J_{3,4}$ 9.4, H-3), 5.43 (1H, dd, $J_{2''',3'''}$ 3.8, $J_{3''',4'''}$ 9.4, H-3'''), 5.48 (1H, dd, $J_{2',3'}$ 3.8, $J_{3',4'}$ 9.4, H-3'),5.50-5.59 (4H, m, H-1', H-1'', H-1''' and H-3'') and 7.18-7.48 (20H, m, 4 x Ph);

^{13}C NMR (125 MHz; $CDCl_3$): δ_C 8.62 (*C*H$_3$CH$_2$N), 20.59, 20.64, 20.66 and 20.71 (*C*H$_3$CO), 45.50 (*C*H$_3$CH$_2$N), 54.98 (CH$_3$O), 61.02 (C-6'''), 61.75 (C-2), 62.34 (d, $^{3}J_{C,P}$ 7.6), 62.41 (d, $^{3}J_{C,P}$ 8.6), 62.59 (d, $^{3}J_{C,P}$ 7.6) (C-2', C-2'' and C-2'''), 63.51 (d, $^{2}J_{C,P}$ 4.1), 64.08 (d, $^{2}J_{C,P}$ 4.1), 64.51 (d, $^{2}J_{C,P}$ 5.1) (C-6', C-6'' and C-6'''), 70.99 (d, $^{3}J_{C,P}$ 9.1, C-5), 71.99 (d, $^{3}J_{C,P}$ 9.9), 72.07 $^{3}J_{C,P}$ 9.6,) (C-5' and C-5''), 72.70, 72.80, 73.00, 73.07 (2C), 73.13, 73.26, 73.41 and 73.55 (C-3, C-3', C-3'', C-3''', C-4, C-4', C-4'', C-4''' and C-5'''), 74.75, 74.77 and 74.91 (Ph*C*H$_2$), 93.86 (d, $^{2}J_{C,P}$ 3.5), 93.94 (2C, d, $^{2}J_{C,P}$ 3.6) (C-1', C-1'' and C-1'''), 98.43 (C-1), 127.46-138.47 (Ph) and 169.69, 169.74, 169.91 and 170.03 (*C*=O);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.25, -3.19 (P and P') and -2.74 (P'').

High resolution ES-MS(-): found *m/z*1547.2569 [M-3Et$_3$N-H]$^-$ ($C_{61}H_{74}N_{12}O_{30}P_3^-$ requires *m/z*1547.3852), 1569.2299 [M-3Et$_3$N-2H+Na]$^-$ ($C_{61}H_{73}N_{12}NaO_{30}P_3^-$requires *m/z*1569.3672), 1585.2056 [M-3Et$_3$N-2H+K]$^-$ ($C_{61}H_{73}KN_{12}O_{30}P_3^-$requires *m/z*1585.3411).

Attempted condensation of the H-phosphonate 89 and the phosphosaccharide 96



A mixture of the H-phosphonate **89** (45 mg, 56 µmol, 1.5 eq.) and the tetrasaccharide triphosphate **96** (66 mg, 36 µmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (10 µl, 84 µmol, 2.3 eq.) was added, and the solution was stirred for at 20 ^{o}C for 40 min. The solution was cooled to -40 ºC prior Et_3N (31 µl, 224 µmol, 6.2 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (28 mg, 112 µmol, 3.1 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, before it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated.

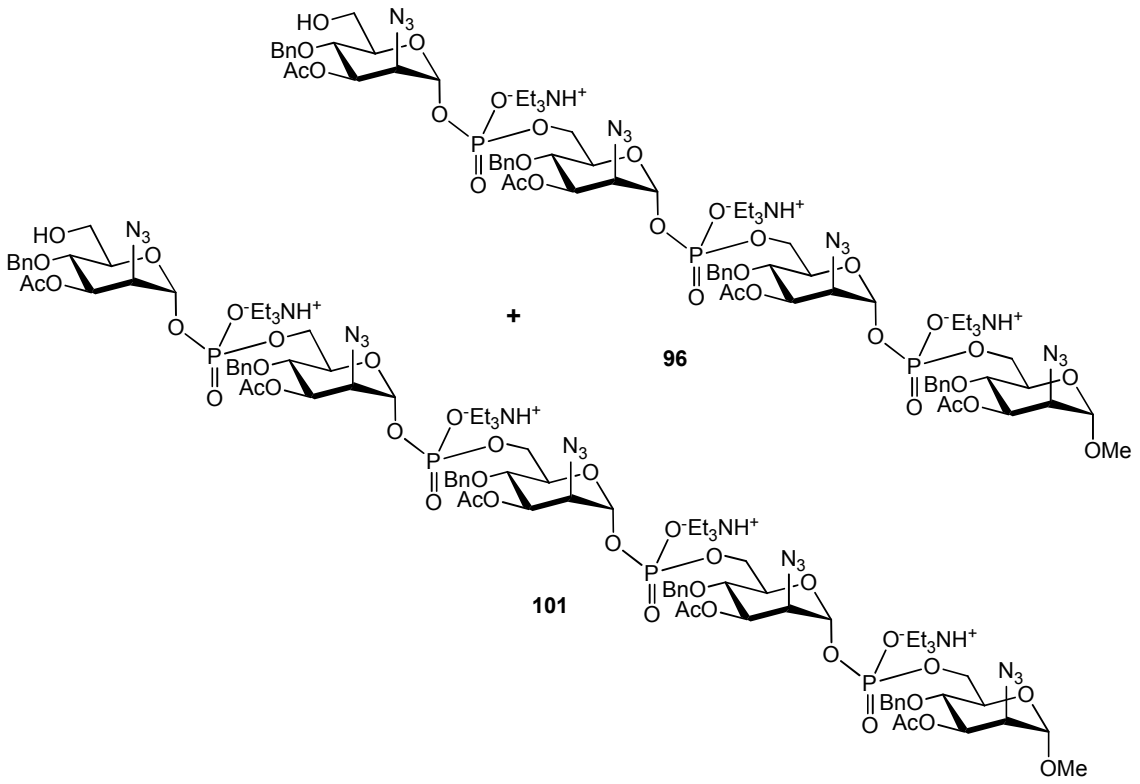
FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→80:20:1)] of the residue gave the product(43 mg)which was identified (using ^{1}H and ^{31}P NMR data) as ~1:1 inseparable mixture of

the pentasaccharide tetraphosphate **100** and the starting material **96**. The mixture was then used in a condensation attempt with the H-phosphonate **89** in a similar conditions, but no further progress was observed, indicating, probably quite low reactivity of the 6'''-OH group in compound **96**. The ~1:1 inseparable mixture of the pentasaccharide tetraphosphate **100** and the starting material **96** was then detritylated with 1% TFA in CH_2Cl_2 in the conditions identical to the preparation of compound **96**. This produced a ~1:1 mixture (39 mg) of the pentasaccharide tetraphosphate **101** and the starting material **96**. Regrettably, this mixture appeared to be also inseparable on silica.

For the mixture **96** and **100**

^{1}H NMR (500 MHz; $CDCl_3$): δ_H (selected signals) 1.94, 1.95, 1.99, 2.01, 2.03 (5 x s, in a ratio of ~1:1:2:3:1, Ac groups of **96** and **100**), 3.32 and 3.33 (2 x s, in a ratio of 1:1, anomeric OMe groups of **96** and **100**), 3.73 (s, C_6H_4O*Me* of **100**), 5.33 (2 x dd, $J_{2,3}$3.8, $J_{3,4}$9.4, H-3 of **96** and H-3 of **100**), 5.68 (dd, $J_{1''',2'''}$1.2, $J_{1''',P}$7.1, H-1'''of **96**), 5.75 (dd, $J_{1'''',2''''}$1.4, $J_{1'''',P}$7.7, H-1''''of **100**) (integration ratio of signals at 5.33, 5.68 and 5.75 is ~2:1:1), 5.43-5.56 (m, H-1', H-1'', H-3', H-3'' and H-3''' of **96** and H-1', H-1'', H-1''', H-3', H-3'', H-3''' and H-3'''' of **100**) and 6.70-7.53 (m, Ph groups of **96** and Ph and C_6H_4 of **100**);

^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.30, -3.19, -3.17, -3.11 (P and P' of **96** and P, P', P'' and P''' of **100**) and -2.86 (P'' of **96**); a ratio of **96** :**100** = ~1:1.



For the mixture **96** and **101**

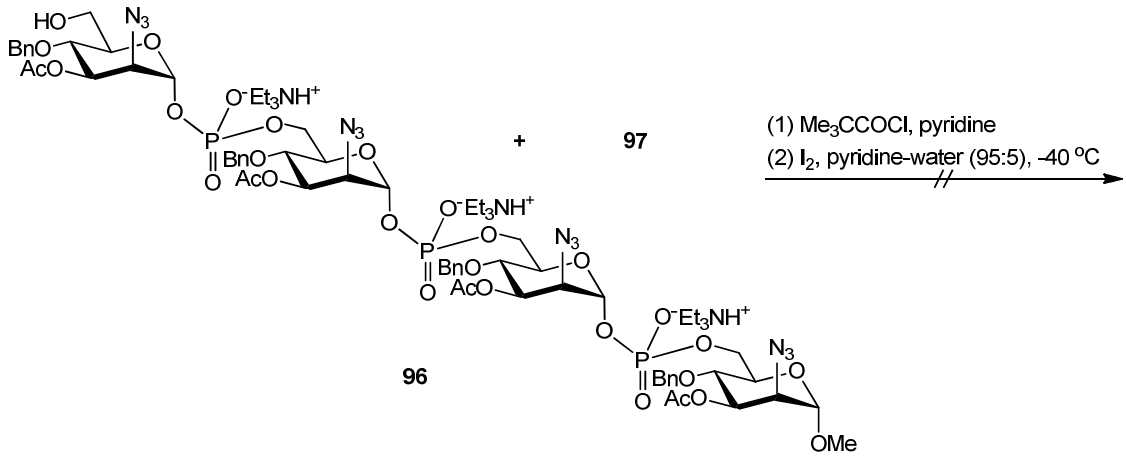
υ_{max} (film)/cm^{-1} (*inter alia*): 2101 (-N_3);

$[\alpha]_D$ +11.7 (*c* 1, $CHCl_3$);

^{1}H NMR (500 MHz; $CDCl_3$): δ_H (selected signals) 1.982, 1.988, 1.991, 1.999, 2.001 and 2.008 (6 x s, in a ratio of ~ 1:2:1:1:1:2, Ac groups of **96** and **101**), 3.32 and 3.33 (2 x s, in a ratio of 1:1, anomeric OMe groups of **96** and **101**), 5.33 (dd, $J_{2,3}$3.8, $J_{3,4}$9.5, H-3 of **96** and H-3 of **101**), 5.43 (dd, $J_{2''',3'''}$3.7, $J_{3''',4'''}$9.3, H-3''' of **96** and $J_{2'''',3''''}$3.7, $J_{3'''',4''''}$9.3, H-3'''' of **101**), 5.47-5.59 (m, H-1', H-1'', H-1''', H-3', H-3'' of **96** and H-1', H-1'', H-1'", H-1'''', H-3', H-3'' and H-3''' of **101**) and 7.19-7.34 (m, Ph groups of **96** and **101**);

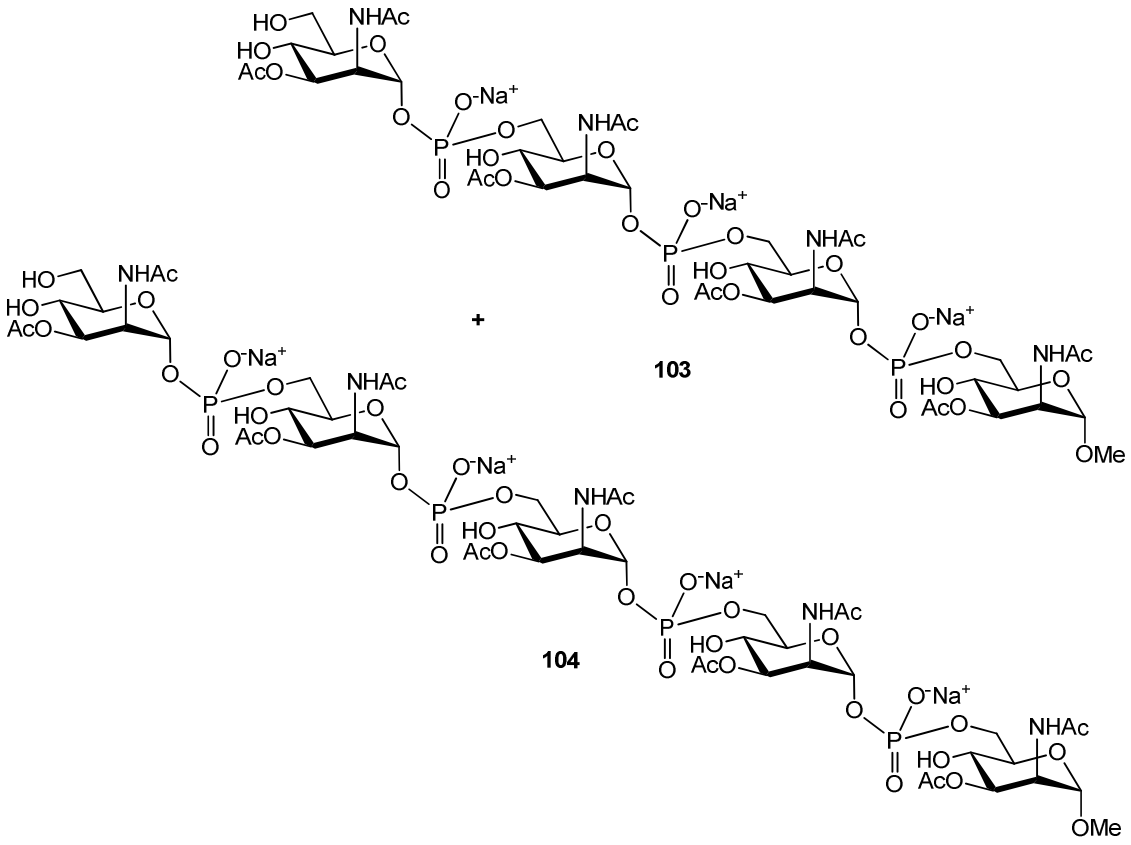
^{31}P NMR (200 MHz; $CDCl_3$): δ_P -3.39, -3.29, -3.19 (P, P' and P'' of **101**), -3.26, -3.19 (P and P' of **96**), -2.75 (P''' of **101**) and -2.74 (P'' of **96**); a ratio of **96** :**101** = ~1:1.

Attempted condensation of the H-phosphonate 97 and the phosphosaccharide 96



A mixture of the H-phosphonate **97** (7.4 mg, 17.8 μmol, 1.2 eq.) and the tetrasaccharide triphosphate **96** (30 mg, 16.2 μmol) was dried by evaporation of pyridine (3 x 3 ml) therefrom. The residue was then dissolved in pyridine (1 ml) under argon, before trimethylacetyl chloride (2.6 μl, 21.3 μmol, 1.5 eq.) was added, and the solution was stirred for at 20 ^{o}C for 30 min. The solution was cooled to -40 ºC prior Et_3N (10 μl, 71.3 μmol, 3 eq.) and a mixture of pyridine-water (95:5; 1 ml) were added. Iodine (9 mg, 35.6 μmol, 1.5 eq.) was then added, the mixture was stirred for 30 min at -40 ºC, before it was allowed to slowly warm to room temperature (~1 h) with stirring. Chloroform was then added to the mixture, and the solution was washed successively with cold 0.5 M $Na_2S_2O_3$ and cold 0.5 M TEAB buffer, dried by filtration through cotton wool and concentrated. FCC [CH_2Cl_2-methanol-Et_3N, (95:5:1→80:20:1)] of the residue gave the starting material phosphosaccharide **96**.

Hydrogenation and N-acetylation of the mixture 96 + 101



A solution of **96** + **101** (39 mg) in MeOH (5ml) was treated with IR-120 (Na^+) resin while stirring for 16 h. The resin was then filtered off and the solution evaporated to dryness. The residue was then dissolved in THF (0.5 ml) and MeOH (3 ml) with the addition of Ac_2O (200 µl) and AcOH (20 µl). The solution was then hydrogenated over $Pd(OH)_2/C$ (50 mg) at ambient temperature for 16 h. The mixture was then filtered through a Celite pad and the filtrate concentrated under reduced pressure to give a mixture of the oligomers **103** and **104** (~1:1, 24 mg) as an amorphous solid.

^{1}H NMR (500 MHz; D_2O): δ_H (selected signals) 1.99-2.06 (m, 8 x Ac groups of **103** and 10 x Ac groups of **104**), 3.31 and 3.38 (2 x s, anomeric OMe groups of **103** and **104**), 3.81-4.27 (m, H-4, H-4', H-4'', H-4''', H-5, H-5', H-5'', H-5''', H-6^a, H-6^b, H-6$^{a'}$**,** H-6$^{b'}$, H-6$^{a''}$, H-6$^{b''}$, H-6$^{a'''}$ and H-6$^{b'''}$ of **103** and H-4, H-4', H-4'', H-4''', H-4'''', H-

5, H-5', H-5'', H-5''', H-5'''', H-6^{a}, H-6^{b}, H-6$^{a'}$**,** H-6$^{b'}$, H-6$^{a''}$, H-6$^{b''}$, H-6$^{a'''}$, H-6$^{b'''}$, H-6$^{a''''}$ and H-6$^{b''''}$ of **104**), 4.44-4.48 (m, H-2 of **103** and H-2 of **104**) 4.50-4.57 (m, H-2', H-2'', H-2''' of **103** and H-2', H-2'', H-2''' and H-2'''' of **104**), 4.69 (m, H-1 of **103** and H-1 of **104**), 4.98-5.03 (m, H-3 of **103** and H-3 of **104**) 5.11-5.18 (m, H-3', H-3'', H-3''' of **103** and H-3', H-3'', H-3''' and H-3'''' of **104**), 5.37-5.44 (m, H-1', H-1'' and H-1''' of **103** and H-1', H-1'', H-1''' and H-1'''' of **104**);

^{13}C NMR (125 MHz; D_2O): δ_C 20.38 (CH_3COO), 21.75 (CH_3CON), 49.96 (C-2 of **103** and C-2 of **104**), 50.71 (br, C-2', C-2'' and C-2''' of **103** andC-2', C-2'', C-2''' and C-2'''' of **104**), 54.93 (CH_3O of **103** and CH_3O of **104**), 59.99 (C-6''' of **103** and C-6'''' of **104**), 63.39, 63.75, 63.85, 64.27 and 64.56 (C-4, C-4', C-4'', C-4''', C-6, C-6' and C-6'' of **103** andC-4, C-4', C-4'', C-4''', C-4'''', C-6, C-6', C-6'' and C-6''' of **104**), 71.02 (d, $^{3}J_{C,P}$ 8.0, C-5 of **103** and C-5 of **104**), 71.89, 71.93, 71.98, 72.15, 72.21, 72.27 and 72.43 (C-3, C-3', C-3'', C-3''', C-5' and C-5'' of **103** andC-3, C-3', C-3'', C-3''', C-3'''', C-5', C-5''and C-5''' of **104**), 73.36 (C-5''' of 1**03** and C-5'''' of **104**), 94.90 (br, C-1', C-1'' and C-1''' of **103** andC-1', C-1'', C-1''' and C-1'''' of **104**), 99.48 (C-1 of **103** and C-1 of **104**), 173.46 (CH_3COO) and 174.50 (CH_3CON);

^{31}P NMR (200 MHz; D_2O): δ_P -2.42 (br, P, P' and P'' of **103** and P, P', P'' and P''' of **104**).

High resolution ES-MS(-). Signals corresponding to the tetrasaccharide triphosphate **103**:

found*m/z* 416.4192 $[M - 3Na]^{3-}$ ($C_{41}H_{64}N_4O_{34}P_3^{3-}$requires *m/z*416.4210), 625.1355 [M - 3Na + H]$^{2-}$ ($C_{41}H_{65}N_4O_{34}P_3^{2-}$requires *m/z*625.1352), 636.1260 [M - 2Na]$^{2-}$ ($C_{41}H_{64}N_4NaO_{34}P_3^{2-}$requires *m/z*636.12615), 1273.2712 [M - 2Na + H]$^{-}$ ($C_{41}H_{65}N_4NaO_{34}P_3^{-}$requires *m/z*1273.2596), 1295.2566 [M - Na]$^{-}$ ($C_{41}H_{64}N_4Na_2O_{34}P_3^{-}$ requires *m/z*1295.2416).

Signals corresponding to the pentasaccharide tetraphosphate **104**:

found*m/z* 393.1652 $[M - 4Na]^{4-}$ ($C_{51}H_{79}N_5O_{43}P_4^{4-}$requires *m/z*393.3280), 524.7719 $[M - 4Na + H]^{3-}$ ($C_{51}H_{80}N_5O_{43}P_4^{3-}$requires *m/z*524.7731), 798.6538 $[M - 3Na + H]^{2-}$ ($C_{51}H_{80}N_5NaO_{43}P_4^{2-}$requires *m/z*798.6543), 809.6487 $[M - 2Na]^-$ ($C_{51}H_{79}N_5Na_2O_{43}P_4^{2-}$ requires *m/z*809.6453), 1642.3367 $[M - Na]^-$ ($C_{51}H_{79}N_5Na_3O_{43}P_4^-$requires *m/z*1642.2798).

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